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Effect of benzyladenine on fruit thinning and its mode of action on 'McIntosh' apples.

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EFFECT OF BENZYLADENINE ON FRUIT THINNING AND ITS MODE OF ACTION ON 'MCINTOSH' APPLES

A Dissertation Presented

by

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DEDICATION

To my parents and my wife for their love, support and patience.

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ABSTRACT

EFFECT OF BENZYLADENINE ON FRUIT THINNING

AND ITS MODE OF ACTION ON 'MCINTOSH' APPLES

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6-benzylaminopurine (BA), which was applied at the 10 mm stage of fruit development at 50 and 100 mg.liter^{-1} to 'McIntosh' apples (Malus domestica Borkh), effectively thinned fruit, and increased fruit size and caused seed abortion. Regardless of BA treatment, there were two distinguishable peaks of fruit abscission during 'June drop' on 'Morespur McIntosh' apples . There was a large peak about 14 days after treatment and a second smaller one 24 days after treatment. The first abscission peak was increased by BA, and approximately half of then existing fruit abscised from treated trees between 10 and 14 days after treatment, whereas only one fourth abscised from the control trees. The number of seeds per fruit was lower in abscising fruit than in persisting fruit regardless of BA treatment. Seed number of abscising fruit was increased linearly by BA application.

BA enhanced the translocation of ^{14}C-sorbitol from leaves to fruit when applied directly to the fruit, but not when applied directly to the leaves. Therefore, it is unlikely that BA thinned apple fruit directly through restricting the translocation of photosynthates moving from leaves to fruit.

Net photosynthesis was decreased and dark respiration was increased when temperature following BA application was high (30°C), whereas there was no effect when temperature was lower (20°C). Total nonstructural carbohydrates, total soluble sugars, reducing sugars, and starch in the leaves decreased dramatically over the 12 or 13 day observation period, regardless of BA treatment. These carbohydrate levels were further lowered by BA application. Abscising fruit, based on specific reddening of pedicel, had higher reducing sugars, total soluble sugars, starch, and total nonstructural carbohydrates. The levels of these carbohydrates in fruit from BA treated trees were similar to that from untreated control trees.

Foliar application of 5% sorbitol solution had no effect on fruit set. Removal of bourse shoot tip increased fruit set regardless of BA treatment. BA thinned fruit regardless of whether shoot tips were removed or not, and there was no interaction between BA application and shoot tipping.

Experiments were conducted to evaluate the effects of BA on fruit set and fruit development on small fruiting branches supported by different numbers of leaves per fruit. BA thinned fruit only when one leaf per fruit was on the girdled small fruiting branch, but not when the leaf number was greater than two. Fruit weight and soluble solids content increased quadratically with increasing leaf number per fruit. BA reduced fruit growth rate when less than 16 leaves were present on the girdled branches between 3 and 7 days after treatment, but it did not affect fruit growth rate when 32 leaves were on the girdled branches. Increasing leaf number also increased normal seed number while decreasing the number of aborted seeds, but it had no effect on the number of total seeds per fruit. BA reduced the number of normal seeds per fruit only when the number of leaves per fruit was less than 4.

These results were discussed in relation to carbohydrate supply and source-sink relationships.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Many apple cultivars are considered biennial and as such they bear a heavy crop one year and a light one the following year (Childers et al., 1995; Harley et al., 1942). In the 'on' year, fruits that are harvested are characterized as being small, having poor quality, and a diminished commercial value (Childers et al., 1995). High levels of gibberellins originating from seeds of developing fruit reduce or completely inhibit flower bud formation for the following year (Hoad, 1978; Luckwill, 1969; Luckwill et al., 1969). In the 'off' year, there is a reduced return bloom, thus few if any fruit. Therefore, it is important to remove excessive fruit from apple trees at an early stage of fruit development to increase fruit size, color, and quality the year of application, and to promote flower bud formation the following year (Childers et al., 1995; Harley et al., 1942; Miller, 1988).

Since the initial suggestion by Mclaughlin and Greene (1984) that benzyladenine (BA) might be a good chemical thinner for apples, there have been a number of reports that have confirmed the effectiveness of BA as a chemical thinner on many apple cultivars (Bound et al., 1991; Elfving and Cline, 1993 a, b; Greene, 1993; Greene et al., 1990; Wismer et al., 1995). In 1994, Accel was registered for use as a chemical thinner for apples (Abbott Laboratories, 1993). It is an altered Promalin formation that contains 90% BA and 10% GA4+7. Our understanding of the mechanism whereby BA acts as a chemical thinner on apples is unclear. Studies to determine the mechanism of action of BA as a chemical thinner

on apples have lagged behind investigation designed to work out the practical details for effective and consistent commercial thinning.

Previous reports showed that BA thins linearly over a wide range of concentrations, but 50 to 100 ppm are generally required to do an effective job (Bound et al., 1991; Byers and Carbough, 1991; Elfving, 1991; Ferree, 1996; Greene and Autio, 1989; Greene et al., 1990; Greene, 1993). BA thins over a wide range of application times and stages of fruit development from bloom to approximately 4 weeks after full bloom. However, the maximum thinning response occurs when BA is applied at the 10-14 mm stage of fruit development , which is frequently about 14 to 21 days after full bloom (Bound et al., 1991; Greene and Autio, 1989; Greene, 1993).

BA, similar to another chemical thinner NAA (Schneider, 1978), thins apple fruits more effectively when applied to the leaves than to the fruit (Greene et al., 1992). The lack of significant thinning can not be attributed to lack of BA absorption by the fruit because considerably more BA enters into the fruit than enters through either surface of the leaf (Greene et al., 1992). It has been suggested that the mechanism of fruit thinning by NAA is due to reduced energy available to developing fruit either by interference with photosynthesis (Stopar et al., 1997) or by the reduced translocation of metabolites, including photosynthates, from leaves to the fruit (Schneider, 1975 a, 1978). BA promotes rather than reduces ^{14}C-assimilate export from treated leaves to developing 'Valencia' orange fruit (Maul et al. 1986). A preliminary experiment showed that BA may increase dark respiration rates of apple leaves, thus reducing net photosynthesis (Greene, Unpublished data).

Carbohydrates have been implicated in fruit set and fruit development. Shading or application of photosynthetic inhibitors shortly after bloom, which decreased photosynthesis

rates and thus reduced the carbohydrates available to the fruitlets, increased early fruit abscission of apple (Auchter et al., 1926; Byers et al., 1985; 1990 a, b; Cohen, 1991; Polomski et al., 1988; Beruter and Droz, 1991; Schneider, 1977, 1978; Doud and Ferree, 1980), peach (Byers et al., 1985; DelValle et al., 1985), sweet orange (Moss, 1976), and litchi (Yuan and Huang, 1988). Byers et al., (1991) reported that even two days of artificial shade induced more apple fruit abscission than NAA, ethephon, or carbaryl+oil spray. They concluded that naturally occurring apple fruit drop in the period from 15 to 40 days after full bloom appeared to be aggravated by 2-4 days of cloudy weather. Fruit from artificially shaded or photosynthetic inhibitor terbacil-treated trees have lower total sugars and reducing sugars than those not treated (Polomskiet al., 1988). Treatments which increase carbohydrate concentration within the apple tree increase fruit set (Greene, 1927; Williams et al., 1980). Injection of sorbitol solutions into branches increases apple fruit set (Dennis, 1986). A droplet of 10% sucrose solution applied daily to bean petiole explants also has been reported to inhibit petiole abscission (Brown and Addicott, 1950).

Removal of spur and shoot leaves causes an extensive fruit abscission (Arthey and Wilkinson, 1964; Ferree and Palmer, 1982, Quinlan and Preston, 1971). A minimum of two leaves per spur are required for normal apple fruit set (Heinicke, 1917). Final fruit set is reduced by reduction in spur leaf area (Ferree and Palmer, 1982).

Fruit abscission is considered to be the result of competition between fruits, and between fruits and vegetative shoots, for essential metabolites (Abbott, 1960; Knight, 1980; Quinlan and Preston, 1971). Abscission from heavily set trees is always greater than from light set trees, indicating that inter-fruit competition is a major component in 'June drop' (Childers

et al., 1995; Denne, 1963; Knight, 1980). Removal of the shoot tip, reduces drop, thereby increasing yield (Quinlan and Preston, 1971).

Carbon balance models indicate a potential limitation of carbon availability during the first five weeks after bloom, a critical period for fruit set and fruit cell division (Lakso and Corelli Crappadelli, 1992). However, Abruzzese et al. (1995) reported that abscised apple fruit had higher level of soluble reducing sugars and sucrose than persisting fruit. Also, abscising citrus fruit had higher reducing sugars and total sugars than persisting fruit (Ruiz and Guardiola, 1994).

The primary objective of this study was to gain a better understanding of the mechanism of action of BA when used as a chemical thinner on apples. The specific objectives of this study were:

1. To evaluate the effect of BA on the translocation of assimilate from leaves to developing fruit in apples.

2. To measure the net photosynthesis rates and dark respiration rates of apple leaves at different temperatures following BA application.

3. To evaluate the influence of BA on the nonstructural carbohydrate status in the leaves and fruits of apples and its relation to the abscission of apple fruit.

4. To investigate the effects of sorbitol and BA on fruit set.

5. To determine the effects of removal of bourse shoot tip and BA on fruit set.

6. To evaluate the effects of different leaf numbers or area and BA on fruit abscission and fruit development.

CHAPTER I I

LITERATURE REVIEW

Removal of excess fruit from apple trees is one of the most important management practices a grower is required to do to produce high quality fruit in commercial apple orchards. Thinning improves fruit size, color, and quality at harvest, and increases return bloom the following year, thereby reducing alternate bearing (Childers et al., 1995; Williams and Edgerton, 1981). Chemical thinning is superior to hand thinning because it is less expensive and it can be done earlier. Therefore, it is possible to get greater fruit size and more reliable return bloom (Childers et al., 1995; Miller, 1988). However, chemical thinning is a difficult practice, and results may be erratic and unpredictable (Looney, 1986). The consequence of insufficient thinning is the potential for major economic loss.

Thinning Chemicals Used

There are several thinning chemicals available for use. Naphthaleneacetic acid (NAA) and carbaryl (sevin) are two of the most frequently used postbloom thinning chemicals. Recently Accel, containing primarily BA, but also 10% GA_{4+7}, was registered for use as a chemical thinner for apple (Abbott Laboratories, 1993).

Carbaryl

Carbaryl is one of the safest chemical thinners and it rarely overthins (Childers et al.,1995; Greene, 1993). It is commonly applied 2-3 weeks after full bloom, at about the 10 mm stage of fruit development, at rates ranging from 100 to 1000 $mg.l^{-1}$ (Knight and Spencer, 1987; McArtney et al., 1995; Williams and Edgerton, 1981). However, it is very

toxic to bees and mite predators (Childers et al., 1995; Greene, 1993), and its use is frequently discouraged in integrated pest management programs (Hislop and Prokopy, 1981).

NAA

NAA is a potent thinner and its thinning response is directly proportional to the amount applied (Childers et al., 1995). To thin effectively, it should be applied at the 8-10 mm stage of fruit development at a concentration of 2.5 to 15 ppm (Leuty, 1973; Williams and Edgerton, 1981). However, overthinning is possible if either cloudy or hot weather immediately follows NAA application (Childers et al., 1995; Greene, 1993; Southwick, 1968). Frequently NAA causes leaf epinasty, and it is not used on some cultivars because it may cause pygmy fruit (Childers et al, 1995; Greene, 1993; Williams and Edgerton, 1981).

Ethephon

Although effective, ethephon is used only in areas where adequate thinning is difficult to achieve using the other chemical thinners (Jonkers, 1979; Jones et al.,1983; Unrath, 1978), because thinning results are too variable (Williams and Edgerton, 1981). Ethephon effects are very temperature dependent, and warm temperature during or following ethephon application may result in overthinning (Childers et al., 1995).

Benzyladenine (BA)

Since the initial suggestion by Mclaughlin and Greene (1984) that BA might be a good chemical thinner for apples, there have been a number of reports that have confirmed the effectiveness of BA as a chemical thinner on 'McIntosh' (Greene and Autio, 1989), 'Empire' (Elfving and Cline, 1993 a, 1993 b; Greene et al., 1990; Wismer et al., 1995), 'Idared' (Elfving, 1989), 'Delicious' (Greene et al., 1990; Greene and Autio, 1994), 'Fuji' (Bound et al., 1991), and other cultivars. BA appears to have several advantages over the other

commonly-used chemical thinners. It increases fruit size as a result of reducing crop load, but in addition, it also increases fruit size and fruit flesh firmness by stimulating cell division, an effect independent of the reduced crop load (Greene et al., 1992; Wismer et al., 1995). It may thin apple fruit more reliably and more consistently than NAA and carbaryl (Elfving and Cline, 1993; Ferree, 1996; Greene, 1993). It appears to have no adverse effects on beneficial mite predators (Thistlewood and Elfving, 1992), and it does not cause severe epinasty commonly observed following NAA application (Greene et al., 1992; Greene, 1993). BA also increases return bloom in many apple cultivars (Bound et al., 1991; Elfving and Cline, 1993 a, 1993 b; Greene et al., 1990).

Factors Affecting the Thinning Results with BA

Many factors affect the thinning effectiveness of BA. BA thins linearly over a wide range of concentrations, but 50 to 100 ppm are generally required to do an effective job (Bound et al., 1991; Byers and Carbough, 1991; Elfving, 1991; Ferree, 1996; Greene and Autio, 1989; Greene et al., 1990; Greene, 1993). BA thins over a wide range of application times and stages of fruit development from bloom to approximately 4 weeks after full bloom. However, the maximum thinning response occurs when BA is applied at the 10-12 mm stage of fruit development , which is frequently about 14 to 18 days after full bloom (Greene and Autio, 1989; Greene, 1993, Bound et al., 1991). Overthinning and undesirable side effects. such as reduced red pigmentation, increased shoot number and total shoot growth. may be caused if concentrations over 150 ppm are used (Greene and Autio, 1990; Greene, 1993; Bound et al., 1991).

The temperature at the time of and following application of thinning chemicals may influence their effectiveness. High temperature causes more thinning (Childers et al., 1995).

Penetration of BA through the adaxial leaf surface is linear with increasing temperature between 15 and 35°C. While penetration of BA similarly increases through the abaxial leaf surface between 15 and 25°C, there is a major increase in penetration between 25 and 35°C. More BA enters the plant through the abaxial leaf surface than through the adaxial surface (Greene, 1993).

When BA is applied to 'McIntosh' and 'Empire' alone or in combination with either carbaryl or NAA, thinning effects are additive and not synergistic (Greene, 1993).

Mechanism of Action of Thinning Sprays

The mechanism(s) whereby chemicals thin apple fruits has been the subject of many experiments and several reviews (Dennis, 1986; Greene, 1993; Looney, 1983, 1986; Williams and Edgerton, 1979, 1981). However, the modes of action of the postbloom thinning chemicals are not entirely known (Williams and Edgerton, 1981).

Mode of Action of NAA

Several hypotheses have been proposed for the mechanism of action of NAA. Struckmeyer and Roberts (1950) suggested that NAA temporarily delayed the abscission of young fruits, which led to greater competition for nutrients between fruitlets, thus increasing fruit abscission. Later, Luckwill (1953) proposed that NAA primarily thinned by increasing seed abortion. These two theories are not generally accepted, because NAA does not always delay abscission, NAA may thin moderately to heavily without delaying fruit drop (Batjer and Billingsley, 1964), and no relationship is found between the number of viable seeds and the thinning effect of NAA (Marsh et al., 1960; Batjer and Thompson, 1961; Williams and Edgerton, 1981). Later, Crowe (1965) reported that NAAm reduced auxin-like substances in the diffusates of apple fruitlets 1 day after treatment. However, Ebert and Bangerth

(1981) measured the levels of both extractable and diffusable hormones in apple fruitlets following application of thinning chemicals (carbaryl, ethephon, NAAm), and found no consistent association between auxin concentration and thinning response.

Ethylene has been implicated in the thinning response following the application of NAA and NAAm. NAA and NAAm stimulate ethylene biosynthesis in treated leaves and fruits (Edgerton, 1981; Walsh et al., 1979; Curry, 1991). Curry (1991) suggested that the mode of action of NAA-induced fruitlet abscission might be a result of ACC being synthesized in the leaf tissue and transported to the site of abscission zone formation where it was converted to ethylene. However, Schneider (1975) found little relation between the thinning effect of NAA and ethylene evolution from apple fruitlets, leaves or pedicels. Ebert and Bangerth (1982) found little or no increase in ethylene following NAAm treatment, despite considerable stimulation of fruit drop.

Others have suggested that the primary mechanism of fruit thinning by NAA is due to reduced energy available to developing fruit either by interference with photosynthesis (Stopar et al., 1997) or by the reduced translocation of metabolites, including photosynthates, from leaves to the fruit (Schneider, 1975 a, 1978). Schneider (1977) suggested that fruit abscission was caused by a series of events which were initiated by reduced metabolites reaching the young fruit. Fruit growth was slowed. which resulted in reduced auxin in the fruit, increased ethylene synthesis, and then initiation of the abscission process and finally abscission. Schneider(1978) reported that NAA thinned 'Golden Delicious' and 'Staymared' apples when applied to leaves, but no thinning was observed when NAA was applied only to the fruit.

Mode of Action of Carbaryl

Considerable progress has been made in determining the mode of action of carbaryl as an apple fruit thinner. Williams and Batjer (1964) reported that carbaryl was more effective when applied to fruits rather than leaves. They suggested that carbaryl interfered with the movement of metabolites within the vascular system which then led to reduced growth of the fruit and eventual abscission. Carbaryl causes seed abortion (Batjer and Westwood, 1960), but no correlation is found between fruitlet thinning and seed abortion (Williams and Batjer, 1964; Williams and Edgerton, 1981; Teubner and Murneek, 1955). However, Knight (1983) reported that application of carbaryl to either fruitlets, pedicels or bourse shoot leaves caused significant fruitlet abscission, but no thinning if applied only to the rosette leaves. He suggested that the mode of action of carbaryl was that it stimulated the activity of existing sinks (bourse shoot), thereby increasing competition and limiting metabolite flow into the fruitlets (Knight, 1983). This suggestion helps explain why carbaryl can promote abscission of only some fruitlets in a cluster. Byers et al.(1990) also reported similar results. Carbaryl usually thins apple fruits without stimulating ethylene production (Landsberg, 1979; Ebert and Bangerth, 1982; Schneider, 1975)).

Mode of Action of Ethephon

Application of ethephon induces abscission, and AVG, an inhibitor of ethylene biosynthesis, inhibits ethylene production and reduces fruit drop (Williams, 1981; Walsh and Faust, 1982). Martin and Brooks (1973) proposed a screening technique for thinning chemicals for peach based on a compound's ability to stimulate ethylene evolution from leaves. However, Ebert and Bangerth (1982) found marked increases in ethylene levels in ethephon-treated fruits, even when drop was not stimulated.

Mode of Action of BA

Little is known about how BA acts as a chemical thinner on apples. BA thins apple fruits more effectively when applied to the leaves than to the fruit (Greene et al., 1992). Since considerably more BA enters into the fruit than enters through either surface of the leaf, the lack of significant thinning can not be attributed to lack of BA absorption by the fruit (Greene et al., 1992). Greene et al.(1992) reported that BA treatments that thinned also increased ethylene production linearly in both leaves and fruit 24 hours after application, but the magnitude of increase was quite small and not considered large enough to be the primary cause for thinning. BA appears to be a nonselective thinner similar to NAA, since it thins spurs with multiple fruit similar to spurs that contain just one fruit (Greene et al.,1992). A preliminary experiment showed that BA may increase dark respiration rates of apple leaves, thus reducing net photosynthesis (Greene, Unpublished data). Net photosynthesis (Pn) is related to P by: $Pn=P-R_d$, where R_d is observed dark respiration rate, P is the difference between the gross leaf photosynthesis rate (P_g) and photorespiration rate (R_p) (Pasian and Lieth, 1989). It seems quite unlikely that BA causes thinning by restricting photosynthate movement into the fruit, since BA enhances rather than reduces ^{14}C assimilate export from treated leaves to developing 'Valencia' orange fruit (Mauk et al., 1986).

Carbohydrates and Fruit Set

Carbohydrates are essential for fruit set and subsequent growth. Goldschmidt and Monselise (1977) suggested that early fruit abscission might be a self-regulatory mechanism within the tree that adjusted crop load to a level where carbohydrate and metabolite supply by the tree could be in balance with crop load. Guardiola et al.(1984) and Schaffer et al.(1985) also concluded that the final set of fruit appeared to be controlled by the capacity

of the tree to supply carbohydrates to the developing fruitlets during the post-anthesis period.

Competition among Fruitlets and between Fruitlets and Vegetative Shoots

Apple fruit abscission after fertilization and during 'June drop' may be caused by competition for essential metabolites among individual fruitlets and between fruitlets and vegetative shoots (Abbott, 1960; Knight, 1980; Landsberg and Brain, 1978; Quinlan and Preston, 1971; Wardlaw, 1968). The 'June drop' is heavier when the number of apple flowers setting fruit is high, and partial flower or fruit removal increases the percentage of flowers setting fruit and of fruit persisting through the 'June drop' (Heinicke, 1917; Knight, 1980). Removal of the shoot tip reduced fruit drop and increased yield (Quinlan and Preston, 1971).However, shoot removal shortly after bloom increases initial fruit set, but also increases the 'June drop' (Quinlan and Preston, 1971; Ferree and Palmer, 1982).

Competition for current assimilates was also suggested as a major factor resulting in the premature abscission of flowers and young fruits in cowpea (Adedipe et al., 1976), snapbean (Mauk and Breen, 1986), soybean (Brun and Betts, 1984), mung beans (Clifford, 1979), pepper (Wien et al., 1989), litchi (Roe et al., 1997; Yuan and Huang, 1988; Yuan and Huang, 1993),and peach (Stembridge and Gambrell, 1972).

Photosynthates and Fruit Set

Shading or application of photosynthetic inhibitors shortly after bloom, which decreased photosynthesis rates and thus reduced the carbohydrates available to the fruitlets, increased early fruit abscission of apple (Auchter et al., 1926; Byers et al., 1985; 1990 a, b; Cohen, 1991; Polomski et al., 1988; Beruter and Droz, 1991; Schneider, 1977, 1978; Doud and Ferree, 1980), peach (Byers et al., 1985; DelValle et al., 1985), sweet orange (Moss, 1976), and litchi (Yuan and Huang, 1988). Even 25% shade during two consecutive cloudy days

promotes fruitlet abscission of apple, indicating that a small reduction of photosynthates available to young fruit may adversely affect fruit set (Schneider, 1978).Shading trees for two to three consecutive days starting 14, 21, and 28 days after full bloom with a 92 percent shade cloth causes serious fruit abscission (Byers et al., 1990; Byers et al., 1991). However, shading earlier or later than the above periods for three days did not influence drop. Shading whole trees is more effective in inducing fruitlet abscission than shading limbs on old trees (Beruter and Droz, 1991; Byers et al., 1990). Fruit dry weight, total sugars, and reducing sugars are decreased by shading and application of photosynthetic inhibitors (Polomski et al., 1988).Treatments which increase carbohydrate concentration within the apple tree increase fruit set (Greene, 1937; Williams et al., 1980). Injection of sorbitol solutions into branches increases apple fruit set (Dennis, 1986). A droplet of 10% sucrose solution applied daily to bean petiole explants also has been reported to inhibit petiole abscission (Brown and Addicott, 1950). Application of nucleotides, which promotes photosynthesis, shortly after bloom may increase fruit set of litchi (Yuan and Huang, 1991).

Leaf and Fruit Set

Removal of spur and shoot leaves causes an extensive fruit abscission (Arthey and Wilkinson, 1964; Llewelyn, 1963, 1968; Ferree and Palmer, 1982). Final fruit set is reduced by reduction in spur leaf area (Ferree and Palmer, 1982). A minimum of two leaves per spur are required for normal apple fruit set (Heinicke, 1917).

Patterns of Carbon Fixation, Partitioning and Utilization

Stored reserves of stems and branches of the apple trees decline rapidly early in the growth season, reach a minimum level later in the summer, subsequently increase to a peak in the autumn, and decline very slowly during the winter (Hansen, 1967, 1971; Hansen and

Grauslund, 1973; Hennerty and Forshey, 1971; Kandiah, 1979 a; Oliveira and Priestley, 1988; Vemmos, 1995). Growth in the early spring depends primarily on the stored reserves, while subsequent growth is more dependent on current photosynthate production (Hansen and Grausland, 1973; Kandian, 1979 b). Fruit growth is supported primarily by the spur leaf canopy during the first month after bloom, when fruit cell division is proceeding (Hansen, 1971, 1977; Lakso et al., 1989; Lakso and Corelli Grappadelli, 1992). Only vigorous fruiting spurs are able to support themselves during this period, whereas normal vigor spurs, spurs with less leaf area, spurs with multiple fruit, or shaded spurs are unable to supply the amount of photosynthates required, thus they must import significant photosynthates from nonfruiting spurs. By comparison, extension shoots utilize their own photosynthates for their own growth and it is not until about 5 weeks after bloom that they export significant photosynthates to other portions of the tree (Corelli Grappadelli et al., 1994). During the first 5 weeks after bloom, the patterns of carbon fixation, partitioning and utilization can influence fruit set and final fruit size by influencing fruit growth rates and cell division (Lakso et al., 1989). Carbon balance models indicate a potential limitation of carbon availability during this critical period (Lakso and Corelli Grappadelli, 1992).

Respiration and Fruit Set

The productivity of apple orchard systems is primary related to light availability, light interception, photosynthesis, respiration, and harvest index (Lakso, 1997; Wunsche et al., 1996). Lakso (1994) reported that the respiratory losses required for growth and maintenance of apple tissues might constitute a substantial limitation on orchard productivity. This is true with other crops also. The increased respiration rate at high temperature, which reduces the soluble carbohydrates level in the leaves and limits available carbohydrate supply, results in

the premature abscission of cyathia in poinsettia (Miller and Heins, 1986), young pods of snap bean (Wittwer, 1954), and young bolls of cotton (Guinn, 1976). Guinn (1976) and Wien et al.(1989) reported that when plants were subjected to low light (decreasing photosynthesis) or high night temperatures (increasing respiration), sugar levels in young bolls of cotton dropped, which led to increased ethylene evolution, thereby increasing fruitlet abscission. However, the biochemical mechanism involved was not known. Extensive fruit drop of apple is closely related to weather conditions, especially high minimum temperatures and a lack of sunshine, around 30 days after full bloom (Kondo et al.,1987). The young apple fruit drop is increased by the high day/night temperature, especially night temperature (Fukui et al., 1984 a, b; Kamakura, 1990; Kondo and Takahashi, 1987), and this increased fruit drop is due to the increased consumption of carbohydrates associated with the raised rates of vegetative growth, respiration and ethylene evolution (Kondo and Takahashi, 1987).

Hormones and Fruit Set

Avery et al.(1979) reported a temporary depression in concentration of soluble carbohydrates in apple trees during flowering and early fruit development. Although carbohydrates were low, Avery et al.(1979) did not consider that these were limiting, and they considered the differences in fruit set and yield were likely to be related to plant hormones rather than to the limitation of carbohydrates. Abruzzese et al. (1995) measured the contents of soluble reducing sugars, amino acids, and protein of persisting and abscising fruit of apple during 'June drop'. They found that there were no significant differences in soluble reducing sugars, amino acids, and protein between the seeds from persisting and abscising fruit, and the concentrations of soluble reducing sugars and sucrose in the cortex of fruit were greater in abscised than in persisting fruit.

Seed and Fruit Set

Seeds are rich sources of hormones (Coombe, 1976; Crane, 1964, 1969; Luckwill, 1951). MacDaniels (1928) reported that the number of seeds in an apple fruit might be the determining factor in its ability to stay on the tree during and after 'June drop'. Fruit size increases with seed number (Heinicke, 1917), and eight seeds were required to achieve maximum size of 'Delicious' apple fruits (Williams, 1977). Abbott (1959) reported that surgical removal of seeds induced abscission, but the effect was significantly reduced after 'June drop'. Some apple fruits whose seeds are removed 4 weeks after petal fall can reach maturity if lanolin containing NAA is applied, whereas all the fruit not treated with NAA abscised (Abbott, 1959). In contrast, Beruter and Droz (1991) demonstrated that when compared with untreated control fruit, removal of seeds increased premature fruit abscission, and cutting the calyx end of a fruit without injuring the seeds similarly increased fruit abscission. They concluded that the effect observed after seed removal might not be related to seed content, but rather to results associated with wounding of the fruit tissue.

Endogenous Hormones, Fruit Set and Abscission

Luckwill (1948, 1953) determined the endogenous levels of hormones in seeds of apples and also followed fruitlet drop at corresponding times. He found that the periods of high auxin-like hormone production correlated with low fruit drop and postulated that fruit abscission in apple was related to the production of an auxin-like hormone in seeds. He further suggests that the hormones produced in the seeds moved through the pedicel, and inhibit induction of fruit abscission. However, Crowe (1958) reported that there was no correlation between extractable auxin-like substances and fruit set in either seeded or parthenocarpic apple cultivars, but the levels of auxin-like substances increased as fruit

growth began. Ebert and Bangerth (1982) observed that the diffusible auxin remained high during the period of "June drop". A strong distal transport of auxin was found in apple pedicels during the 'June drop' (Ebert and Bangerth, 1982, 1985).

The role of ethylene in controlling fruit set and abscission of apple has been studied. The ethylene levels are high in the flowers, and then decline in developing fruitlets (Blanpied, 1972; Ebert and Bangerth, 1982). There is no consistent rise in evolution of ethylene prior to or during "June drop" and there are also no consistent differences in ethylene level between abscising and adhering fruitlets during the "June drop" (Blanpied, 1972; Ebert and Bangerth, 1982; Walsh and Solomos, 1983, 1987). Furthermore, McDonnel and Edgerton (1970) reported that daminozide tended to reduce apple fruit set when applied after bloom even though it apparently reduced ethylene evolution.

The early fruit drop process under high night temperature in 'McIntosh Red' apples is divided into four stages (Fukui et al., 1984 a, b): Stage A, when potential fruit drop is indicated by a reduced rate of fruit growth; Stage B, two days after potential fruit drop; Stage C, four days after potential fruit drop; Stage D, abscission. No difference in indole derivative concentration (Fukui et al., 1984 a) or ethylene formation (Fukui et al.,1984 b) was noted between fruits at stage A and persistent fruits; only in stages B and C do potential drop fruits have a higher rate of ethylene evolution than persisting fruit. The cytokinin activity in the seeds of potential drop fruit is only 2.5 to 6.7 percent that of persistent fruit, indicating that a decline in cytokinin activity may be closely related to early fruit drop (Fukui et al., 1985).

High levels of hormones in the seeds are thought to cause diversion of metabolites to the fruit and enable it to compete more efficiently with other growing organs of the plant (Crane, 1964,1969). Beruz and Droz (1991) and Addicott (1982) also reported that the

relatively high concentration of hormonal substances in the growing seeds might be linked to a high metabolic activity of the growing seeds.

CHAPTER III

MATERIALS AND METHODS

All field experiments were conducted at the University of Massachusetts Horticultural research Center in Belchertown, Massachusetts.

^{14}C-Assimilate Movement

Experiment 1-1995

The objective of this experiment was to evaluate the influence of BA on the movement of assimilate from leaves to young growing fruit. In 1995, fruiting spurs were excised, placed in water, and transported to the laboratory. In the laboratory, thirty six uniform spurs with 3 leaves and a 10 mm size fruit were selected, and placed in individual flasks with distilled water at room temperature. A randomized complete block design with 10 replications was used. Leaves on one group of 10 spurs and fruit on another group of 10 spurs were dipped into 100 mg.liter^{-1} BA solution containing 0.1% X-77 surfactant. The third group of 10 spurs were dipped into the solution of 0.1% X-77 surfactant and served as a control. After the BA solution dried, a circle 2 cm in diameter was outlined with dots of India ink on the abaxial surface of one leaf on each spur. Sixteen µl ^{14}C-sorbitol (0.8 µci) were applied within the marked area on the abaxial surface of the leaf using a microsyringe attached to a repeating dispenser (Hamilton, Model PB 600-1). After 24 hours, spurs were harvested and separated into fruit, stem, 2 cm marked portion of the treated leaf, the remaining treated leaf, and the other untreated leaves. The treated leaf disks were each rinsed with 5 ml of water containing 0.1% X-77 surfactant and then 5 ml of distilled water. The

samples were sliced, dried at 70°C, weighed and oxidized for 5 minutes in a biological material oxidizer (Model OX-400; Harvey Instrument Corp., Hillside, N. J.). Evolved $^{14}CO_2$ was collected in an external trap containing 15 ml of Harvey ^{14}C cocktail. Radioactivity was determined by a liquid scintillation counter (Beckman, Model 3801). Counts were adjusted to account for quenching and for biological oxidizer efficiency.

Experiment 2-1996

The objective of this experiment was to further examine the influence of BA on the movement of assimilate from leaves to young growing fruit. In 1996, fruiting spurs were excised, placed in water, and transported to the laboratory. In the laboratory, thirty six uniform spurs with 3 leaves and a 10 mm size fruit were selected, and placed in individual flasks with distilled water at room temperature. A randomized complete block design with 6 replications was used. Leaves on one group of 12 spurs and fruit on another group of 12 spurs were dipped into 100 mg.liter^{-1} BA solution containing 0.1% X-77 surfactant. The third group of 12 spurs were dipped into the solution of 0.1% X-77 surfactant and served as a control. After the BA solution dried, a circle 2 cm in diameter was outlined with dots of India ink on the abaxial surface of one leaf on each spur. Sixteen µl ^{14}C-sorbitol (0.8 µci) were applied within the marked area on the abaxial surface of the leaf using a microsyringe attached to a repeating dispenser (Hamilton, Model PB 600-1). After 24 and 48 hours, spurs were harvested and separated into fruit, stem, 2 cm marked portion of the treated leaf, the remaining treated leaf, and the other untreated leaves. The treated leaf disks were each rinsed with 5 ml of water containing 0.1% X-77 surfactant and then 5 ml of distilled water. The samples were sliced, dried at 70°C, weighed and oxidized for 5 minutes in a biological material oxidizer (Model OX-400; Harvey Instrument Corp., Hillside, N. J.). Evolved $^{14}CO_2$

was collected in an external trap containing 15 ml of Harvey ^{14}C cocktail. Radioactivity was determined by a liquid scintillation counter (Beckman, Model 3801). Counts were adjusted to account for quenching and for biological oxidizer efficiency.

Photosynthesis and Respiration

Experiment 3-1995

The objective of this experiment was to monitor the effects of BA on net photosynthesis and dark respiration of leaves. In 1995, twenty four young 'McIntosh'/M.7 apple trees located outside the greenhouse of Bowditch Hall, University of Massachusetts were blocked based upon vigor into 8 groups (replications) of 3 trees each. A randomized complete block design was used. One uniform fully expanded leaf (the fifth or sixth from the shoot apex) with good light exposure was tagged. One tree in each replication received a dilute spray of either 50 or 100 mg.liter^{-1} BA. One tree in each replication was not sprayed and served as a control. CO_2 exchange rate was measured using a portable photosynthesis system (Model LI-6000; LI-COR, Lincoln, Neb.) equipped with a 250 ml leaf chamber (total system gas volume =390 ml). Leaves were inserted into the leaf chamber. The measurements were made at 0, 1, 3, 6, and 8 days after BA application. Pn and dark respiration of leaves were measured between 9:30 and 12:30 on clear, sunny days, except for the cloudy day at 6 days after treatment. Black cloth was used to cover the leaf chamber to measure dark respiration.

Experiment 4-1996

The objective of this experiment was to further examine the effects of BA on net photosynthesis and dark respiration of leaves. In 1996, twenty four young 'McIntosh'/M.7 apple trees located outside the greenhouse of Bowditch Hall, University of Massachusetts

were blocked based upon vigor into 8 groups (replications) of 3 trees each. A randomized complete block design was used. Two uniform fully expanded leaves (the fifth or sixth from the shoot apex) with good light exposure were tagged. One tree in each replication received a dilute spray of either 50 or 100 mg.liter^{-1} BA. One tree in each replication was not sprayed and served as a control. CO_2 exchange rate were measured using a portable photosynthesis system (Model LI-6200; LI-COR, Lincoln, Neb.) equipped with a 250 ml leaf chamber (total system gas volume =390 ml). Leaves were inserted into the leaf chamber. The measurement was made at 0, 1, 6, and 8 days after BA application. Pn and dark respiration of leaves at high temperature were measured between 9:30 and 12:30 on clear, sunny days, and the dark respiration of leaves at low temperature was measured at 12:00 midnight (the temperature was about 20°C). Black cloth was used to cover the leaf chamber to measure the dark respiration.

Fruit Thinning , Abscission, and Nonstructural Carbohydrate Status

Experiment 5-1995

The objective of this experiment was to observe the effect of BA application on fruit thinning and nonstructural carbohydrate levels in leaves and fruits. In 1995, twenty-four mature 'Morespur McIntosh'/M.7 apple trees were selected and grouped based upon vigor and blossom cluster number into 8 blocks of 3 trees each. A randomized complete block design was used. Two limbs on each tree, 12 to 15 cm in diameter, were tagged and all blossom clusters were counted before bloom. One tree in each block received a spray of BA (ABG-3062, Abbott Laboratory, North Chicago, IL) at either 50 or 100 mg.liter^{-1} on June 1, 1995, at the 10 mm stage of fruit development. One tree in each block was not sprayed and

served as control. All fruit remaining on tagged limbs were counted at the end of the June drop period in July. Fruit characteristics and quality were evaluated at commercial harvest.

Fifteen fruits and twenty spur leaves were collected from each tree at 0, 5, 8, and 12 days after BA application, and were immediately put in a container with dry ice and transported to the laboratory. Leaf area was measured with a LI-COR model LI-3000 area meter. Then all the samples were dried in a forced-air oven at 70°C, weighed, ground in an electric grinder with a 40-mesh screen and used for analysis of reducing sugar, total sugar and starch. Specific leaf weight (SLW) was calculated for the same leaf.

Experiment 6-1996

The objective of this experiment was to further examine the effect of BA application on fruit thinning, seed number, and nonstructural carbohydrate levels in leaves and fruits. In 1996, twenty-four mature 'Morespur McIntosh'/M.7 apple trees were selected and grouped based upon vigor and blossom cluster number into 8 blocks of 3 trees each. A randomized complete block design was used. Two limbs on each tree, 12 to 15 cm in diameter, were tagged and all blossom clusters were counted before bloom. One tree in each block received a spray of BA (ABG-3062, Abbott Laboratory, North Chicago, IL) at either 50 or 100 $mg.liter^{-1}$ on May 30, 1996, at the 10 mm stage of fruit development. One tree in each block was not sprayed and served as control. All fruit remaining on tagged limbs were counted at the end of 'June drop' in July. Fruit characteristics and quality were evaluated at commercial harvest.

Fifteen fruits and twenty spur leaves were collected from each tree at 0, 5, 9, and 13 days after BA application, and were immediately put in the container with dry ice and transported to the laboratory. Leaf area was measured with a LI-COR model LI-3000 area

meter. Then all the samples were dried in a forced-air oven at 70°C, weighed, ground in an electric grinder with a 40-mesh screen and used for analysis of reducing sugar, total sugar and starch. Specific leaf weight (SLW) was calculated for the same leaf.

Thirty persisting and thirty abscising fruit, which were starting to show signs of abscission as evidenced by a yellowing pedicel, were collected from each tree 13 days after BA application. The seeds of each fruit were counted.

Experiment 7-1997

The objective of this experiment was to evaluate the effects of BA on fruit thinning and time course of abscission, and to investigate any difference in nonstructural carbohydrates between persisting and abscising fruit. In 1997, eight mature 'Morespur McIntosh'/M.7 apple trees were selected and grouped based upon vigor and blossom cluster number into 4 blocks of 2 trees each. A randomized complete block design with was used. Two limbs on each tree, 12 to 15 cm in diameter, were tagged and all blossom clusters were counted before bloom. One tree in each block received a spray of BA (ABG-3062, Abbott Laboratory, North Chicago, IL) at 100 $mg.liter^{-1}$ on June 6, 1997, at the 10 mm stage of fruit development. One tree in each block was not sprayed and served as control. Fruit on tagged limbs were counted just before BA application and then fruit remaining on tagged limbs were counted every 3 or 4 days.

Two types of fruit were collected 12 days after BA application: persisting fruit, and abscising fruit which were starting to show signs of abscission as evidenced by a yellowing pedicel. Fifteen fruits were collected from each tree, and weighed, and their equatorial diameter was measured. Then all the samples were dried in a forced-air oven at 70 °C,

weighed, ground in an electric grinder with a 40-mesh screen and used for analysis of reducing sugar, total sugar and starch.

Because the abscising fruit reached the same diameter as that of persisting fruit 12 days later, abscising fruit were sampled 24 days after BA application to compare the considered parameters in persisting vs. abscising fruit with the same diameter.

Sorbitol Application

Experiment 8-1995

The objective of this work was to determine the effect of BA on fruit set following application of sorbitol. In 1995, eight mature 'Morespur McIntosh'/M.7 apple trees were used, and four uniform limbs per tree, 12 to 15 cm in circumference, were tagged. A randomized complete block design was used. Sorbitol at 5% and BA at 100 $mg.liter^{-1}$ were applied alone and in combination on three of the limbs on June 1, 1995, at the 10 mm stage of fruit development, and the fourth limb served as a control. Each tree was a replication. Fruit on each tagged limb were counted just before treatments and at the end of 'June drop', and fruit quality was determined at harvest.

Bourse Shoot Tip Removal

Experiment 9-1996

The objective of this work was to determine the effect of BA on fruit set following removal of bourse shoot tips. In 1996, eight mature 'Morespur McIntosh'/M.7 apple trees were used, and four uniform limbs per tree, 12 to 15 cm in circumference, were tagged. A randomized complete block design was used. Removal of bourse shoot tips and BA at 100 $mg.liter^{-1}$ were applied alone and in combination on three of the limbs on May 30, 1996, at the 10 mm stage of fruit development, and the fourth limb served as a control. Each tree was

a replication. Fruit on each tagged limb were counted just before treatments and at the end of 'June drop' and fruit quality was determined at harvest.

Leaf Number

Experiment 10-1996

The purpose of this experiment was to evaluate the effects of BA on fruit set and development on spurs supported by different leaf areas. In 1996, thirty small fruiting branches of similar vigor were selected and tagged in five mature 'Morespur McIntosh'/M.7 trees. From each small branch a 0.5 cm wide strip of phloem, cambial tissue, and connected bark was removed. The girdle was monitored periodically for cambial growth and was scraped to prevent phloem re-connection until the end of June drop. A randomized complete block design with 15 replications was used. Treatments were 1 fruit with 25 or 50 leaves per branch either untreated or receiving 100 mg.liter^{-1} BA applied on May 30, 1996, at the 10 mm stage of fruit development. Fruit remaining on each tagged branch were counted and fruit diameter was measured after 'June drop'. The fruit quality was determined at harvest.

Experiment 11-1997

The purpose of this experiment was to further examine the effects of BA on fruit set and development on spurs supported by different leaf areas. One hundred and ninety-two small fruiting branches of similar vigor were selected and tagged in eight mature 'McIntosh' trees. From each small branch a 0.5 cm wide strip of phloem, cambial tissue, and connected bark was removed, and then the exposed ring was covered with plastic tape. The girdle was monitored periodically for cambial growth and was scraped to prevent phloem re-connection until the end of June drop. A randomized complete block design with 16 replications was used. Treatments were 1 fruit with 1, 2, 4, 8, 16, or 32 leaves per branch either untreated or

receiving 100 mg.liter^{-1} BA applied on June 6, 1997, at the 10 mm stage of fruit development. Fruit remaining on each tagged branch and fruit diameter were monitored every two or three days after treatment. Leaf area was measured using an area meter (Model LI-3000, LI-COR, Lincoln, Neb.), and the fruit quality was determined at harvest.

Biochemical Analyses

Measurement of Reducing Sugars, Total Sugars and Starch

1. Extraction of Plant Material for Reducing and Total Sugars

The method of Belding and Young (1987) was modified to extract plant material for soluble sugars (reducing sugars and total sugars). The dry plant material was ground using an electric grinder with a 40-mesh screen. Two hundred and fifty mg of ground samples were placed in centrifuge tubes, and 5 ml of 80% ethanol was added to the tubes and thoroughly mixed. The tubes then were covered with aluminium foil and placed in a water bath at 95°C and boiled for 5 minutes. After centrifugation at 13,800 g, the supernatant was pipetted to a 25 ml volumetric. The plant material was re-extracted twice in the same way. The supernatants were combined and brought to 25 ml with distilled water. The solid residue was used for estimation of starch. Six ml of the supernatant was placed in a centrifuge tube and decolorising charcoal (100-120 mg) was added. After mixing, the mixture was centrifuged at 13,800g. The decolorised supernatant was used for determination of reducing sugars and total sugars.

2. Estimation of Reducing and Total Sugars

The method of Flood and Priestley (1973) was used to measure reducing and total sugars. Two ml of the buffered ferricyanide solution, 0.1 ml of supernatant, and 1.9 ml water were pipetted into a test tube. The tubes were covered with foil, heated in a boiling water

bath for 15 minutes, cooled and 2 ml of the arsenomolybdate reagent was added. After at least 5 minutes, the contents were diluted to 25 ml and the green-blue color was measured with a spectrophotometer (PMQ II, Carl Zeiss Oberkochen/Wurtt, Germany) at 740 nm. Standard curves were established using several concentrations of glucose. Absorbent values were converted to mg of glucose per gram of dry mass.

For the determination of total sugars, ten ml of potassium ferricyanide was mixed with 50 ml of buffer III (84.4 g of dipotassium phosphate and 196 g of trisodium phosphate/liter) and diluted to 100 ml with water. One ml of oxalic acid solution, 0.1 ml supernatant, and 1.9 ml water were pipetted into a tube, which was then covered with foil and heated in a boiling water bath for 1 hour. After cooling, two ml of the buffered ferricyanide solution was added and the tube was returned to the bath for 15 minutes. The determination was as described for reducing sugars.

3. Estimation of Starch

The method of Dekker and Richards (1971) was modified to extract starch. One ml of 0.5 N NaOH was added to the solid residue, thoroughly mixed and left for 15-30 minutes. The solution was neutralized with 2 N acetic acid (0.55 ml). One ml amyloglucosidase (Sigma) solution in acetate buffer was added and the solutions was mixed well by shaking. The tube was then covered with aluminium foil to prevent evaporation and heated at 55°C for 4 hours in a water bath.

After the enzymic breakdown of starch to glucose, 15-20 mg decolorising charcoal was added to the solution which was then centrifuged at 12,800 g for 5 minutes. A 0.11 ml aliquot of the supernatant was pipetted into a test tube and neutralized with 0.18 ml 0.5 N

NaOH. The glucose concentration produced from the enzymic hydrolysis of starch was determined as described for reducing sugar.

Statistical Analysis

Trees selected for experiments were as uniform as possible. Statistical analyses included analysis of variance, Duncan's new multiple range test, and orthogonal polynomial comparison, where interaction of main effects were significant, sums of squares were partitioned into the sums of squares of one main effect nested within each level of the other main effect involved in the interaction.. Statistical Analysis Systems Software for PC (SAS Institute Inc., Cary, NC) was used to analyze these data.

CHAPTER IV

RESULTS

^{14}C-Assimilate Movement

Experiment 1-1995

Compared with spurs receiving no treatment, more ^{14}C-sorbitol was translocated from the leaves to the fruit when BA was applied directly to the fruit in a spur (Figure 1). However, there was no difference between ^{14}C-sorbitol movement from leaves to fruit on untreated spurs and on spurs where BA was applied to the leaves.

Experiment 2-1996

Similarly, in the second year movement of ^{14}C-sorbitol was greatest from leaves to fruit on spurs where the fruit were treated with BA, and there was no difference in ^{14}C-sorbitol movement from leaves to fruit between untreated spurs and those on which only the leaves were treated (Figure 2). Overall, more ^{14}C-sorbitol moved from the leaves to fruit in 48 hours than in 24 hours, regardless of treatments.

Photosynthesis and Dark Respiration

Experiment 3-1995

Net photosynthesis rates of apple leaves treated with BA at either 50 or 100 mg.liter^{-1} were significantly lower than that of the control leaves either 1 or 3 days after application, but not 6 or 8 days after treatment (Figure 3). It was a cloudy day at 6 days after treatment, thus all photosynthesis measurements were low. Photosynthesis rates of apple leaves treated BA at 100 mg.liter^{-1} were significantly less than those of leaves treated with 50 mg.liter^{-1}

only at 3 days after treatment. BA at 50 or 100 mg.liter^{-1} significantly increased dark respiration rates of apple leaves only 1 and 3 days after treatment (Figure 4). BA had no effect on the sum of Pn and dark respiration of 'McIntosh' apple leaves (Figure 5).

Experiment 4-1996

Foliar application of BA at 50 or 100 mg.liter^{-1} significantly decreased net photosynthesis rates of apple leaves at 1 and 6 days after treatment, but not at 8 days after treatment (Figure 6). Both rates of BA reduced net photosynthesis comparably.

At 30°C, these treatments correspondently increased dark respiration of apple leaves 1 and 6 days after treatment (Figure 7). However, dark respiration rates of apple leaves treated with BA at 50 or 100 mg.liter^{-1} recovered to the control rate by 8 days after treatment. Dark respiration rates of apple leaves were similar between BA treatments.

Dark respiration rates of apple leaves at 20°C were similar between control and BA treated leaves (Figure 8). Dark respiration rates of apple leaves were considerably higher at 30°C than at 20°C regardless of BA treatment (Figure 7, 8). The sum of Pn and dark respiration of 'McIntosh' apple leaves was not influenced by BA application (Figure 9).

Fruit Thinning, Abscission, and Nonstructural Carbohydrate Status

Experiment 5-1995

BA thinned 'McIntosh' apples linearly with concentration (Table 1). Fruit weight at harvest were increased with increasing concentrations of BA. Flesh firmness and soluble solids content at harvest were not influenced by BA application. BA linearly increased the number of aborted seeds per fruit, but it had no significant effect on the number of normal seeds or the number of total seeds per fruit. Red color was decreased linearly with increasing BA concentration.

Specific leaf weight increased gradually over the 12-day observation period regardless of treatment (Figure 10). Foliar application of BA had no effect on specific leaf weight. Fruit dry weight increased rapidly over time (Figure 11), and was not influenced by BA treatment until the last sample date.

BA reduced total nonstructural carbohydrates, total soluble sugars and starch in the apple leaves 5 and 8 days after treatment, but this difference disappeared 12 days after treatment (Figure 12 B, C, and D). Reducing sugars were lowered in the leaves on BA treated trees only 5 days after treatment (Figure 12 A). Generally, nonstructural carbohydrate contents in the leaves decreased rapidly with time.

Total nonstructural carbohydrates, total soluble sugars, reducing sugars, and starch in the fruit of BA treated trees and control trees were similar (Figure 13 A, B, C, and D).

Experiment 6-1996

BA reduced crop load linearly with concentration on 'McIntosh' apples (Table 2). There was a linear increase in fruit weight at harvest with increasing concentrations of BA. BA did not influence flesh firmness and soluble solids content at harvest, but it reduced red color. At commercial harvest, the number of aborted seeds was increased linearly with increasing concentrations of BA, but the number of normal seeds and total seeds was not influenced by BA application. These results closely compare to those obtained in 1995.During ' June drop', seed number of abscising fruit was increased linearly by BA treatment (Table 3). In persisting fruit, BA linearly decreased the number of normal seeds, increased the number of aborted seeds, but had no effect on the number of total seeds. The number of total seeds was higher in persisting fruit than in abscising fruit, regardless of BA application.

Specific leaf weight increased with time in both BA treated and control trees (Figure 14). Fruit dry weight also increased over time (Figure 15). However, neither the specific leaf dry weight nor fruit dry weight was affected by BA application.

Total nonstructural carbohydrates, total soluble sugars, reducing sugars, and starch in the leaves decreased dramatically with time regardless of BA treatment (Figure 16 A, B, C, and D). The total nonstructural carbohydrates, total soluble sugars and reducing sugars were considerably lower in leaves of BA-treated trees than those of the control trees at 5 and 9 days after treatment. Only reducing sugars were lower 13 days after treatment. BA had no significant influence on starch levels in leaves.

In general, total nonstructural carbohydrates, total soluble sugars, starch, and reducing sugars in the fruit increased with time (Figure 17 A, B, C and D). BA did not significantly affect contents of these in the fruit. However, BA at 100 mg.liter^{-1} reduced starch in fruit 9 days after treatment.

Experiment 7-1997

BA effectively thinned 'McIntosh' apples (Table 4). BA increased fruit weight at harvest by about 60%.

There were two distinguishable fruit abscission peaks during 'June drop' in 'Morespur McIntosh' apples regardless of BA treatment, with a big and a small fruit abscission peak occurring at 14 and 24 days after treatment, respectively (Figure 18). About 54% of then existing fruit on BA treated trees abscised from 10 to 14 days after BA treatment, compared with 25% fruit abscission on control trees.

On June 18, 1997, at 12 days after BA application, the average fruit size of abscising fruit was 32% smaller than that of persisting fruit, whereas the fruit size of the fruit abscising

12 days later (June 30) was similar to persisting fruit (June 18) (Table 5). Abscising fruit the same age had lower fresh weight and dry weight than persisting fruit. Persisting fruit had greater fresh weight but lower dry weight than abscising fruit of similar size. Persisting fruit had higher water content than abscising fruit either 12 or 24 days after application.

Concentrations of reducing sugars, total soluble sugars, and total nonstructural carbohydrates were greater in abscising fruit than in persisting fruit (Table 6).The content of starch was highest in abscising fruit the same size as persisting fruit, whereas starch content was similar in persisting fruit and abscising fruit at the same harvest date.

Sorbitol Application

Experiment 8-1996

BA effectively thinned fruit, and increased fruit weight, aborted seed number and soluble solids contents, but had no significant effect on red color, flesh firmness, normal and total seed numbers (Table 7). The only parameter that sorbitol influenced was to increase fruit weight. There was an interaction between sorbitol and BA treatment only on the number of normal seeds.

Bourse Shoot Tip Removal

Experiment 9-1996

Removal of the bourse shoot tip significantly increased fruit set regardless of BA treatment (Table 8). The number of normal seeds per fruit after removal of bourse shoot tip was higher than that of the control. There was no response to the removal of bourse shoot tip in fruit weight, red color, flesh firmness, soluble solids content, and the number of aborted and total seeds per fruit.

BA effectively thinned 'McIntosh' apples regardless of whether or not bourse shoot tips were removed (Table 8). Fruit weight was increased by BA application, whereas red color was slightly decreased. BA did not influenced flesh firmness and soluble solids content. BA increased the number of aborted seeds, decreased the number of normal seeds, but had no effect on the number of total seeds.

Leaf Number

Experiment 10-1996

BA treatment did not thin fruit when the leaf number per fruit was either 25 or 50 (Table 9). Fruit weight was increased both by BA and by treatment with 50 leaves per fruit. However, there was no significant effect of BA or leaf number on flesh firmness, soluble solids content, and normal, aborted, and total seed number.

Experiment 11-1997

Leaf area increased quadratically with increasing leaf number per fruit (Table 10). Overall, fruit abscission decreased with increasing leaf number per fruit, but BA increased fruit abscission. There was a significant interaction between BA and leaf number per fruit. BA increased fruit abscission only when there was just one leaf per fruit.

At commercial harvest, the only effect BA had was that it reduced the number of normal seeds per fruit (Table 11). Soluble solids content and fruit weight increased quadratically with increasing leaf number per fruit. The larger the number of leaves per fruit, the greater the normal seed number at harvest. There was a BA×leaf number interaction. BA reduced normal seed number on fruit with 1, 2, and 4 leaves and did not affect seed number if more leaves were present. Increasing leaf number per fruit also decreased the number of

aborted seeds while increasing the number of normal seeds. There was no effect of increasing leaf number per fruit on total seed number.

Fruit growth rate was increased with increasing leaf number per fruit during the first three days after treatment. The effect was not influenced by BA treatment (Figure 19 A). Fruit growth rate was also increased with increasing leaf number per fruit from 3 to 7 days after treatment, but low leaf number per fruit dramatically reduced the fruit growth rate, regardless of BA application (Figure 19 B, and C). BA significantly reduced fruit growth rate on all branches except for that with 32 leaves per fruit.

Between 7 and 19 days after BA application, increasing leaf number continued to increase fruit growth rate (Figure 19 D, E, and F). BA application decreased fruit growth rate only when the leaf number per fruit was one. Most fruit abscission occurred during this period. Fruit growth rate continued to be similarly affected by the number of leaves per fruit between 19 and 35 days after BA application (Figure 20 G and H). BA application appeared to have no additional influence on fruit growth rate during this period.

Time course of fruit growth rate is shown in Figure 21. The trend of fruit growth rate of treatment with 8 or 16 leaves per fruit over time and their response to BA were similar to those treated with 4 leaves per fruit. Therefore, only data for treatment with 1, 2, 4, and 32 leaves are presented. Solar radiation and maximum air temperature affected fruit growth rate on all branches, especially on branches with less than 4 leaves, regardless of BA application. Fruit had higher growth rate when the solar radiation and air temperature were relatively high. Fruit growth rate on branches with less than two leaves per fruit was low and fluctuated dramatically over time. BA did not reduce fruit growth rate during the first three

days after treatment when the air temperature was low, but it reduced fruit growth rate between 3 and 7 days after treatment when the temperature was high.

Fruit size was increased with increasing leaf number per fruit at 12 and 19 days after treatment (Figure 22). BA decreased fruit size when the leaf number per fruit was one or two, whereas there was no effect of BA on fruit size when the leaf per fruit was more than four at 12 and 19 days after treatment. At 35 days after treatment, there was a significant increase in fruit size with increasing leaf number per fruit, especially a dramatic increase from one to eight leaves per fruit.

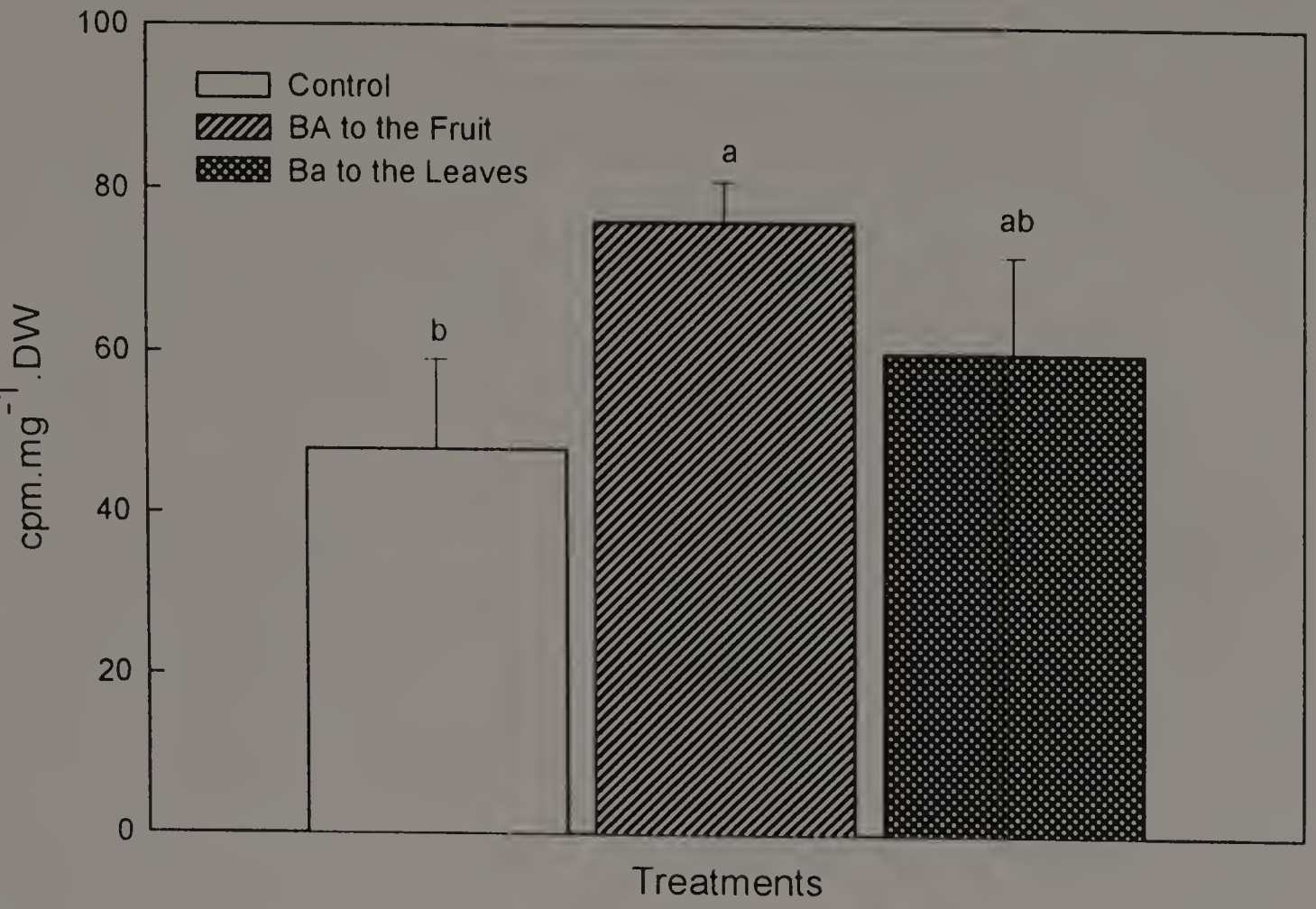

Figure 1. Effects of BA Application to Leaves or Fruit on the Translocation of ^{14}C-Sorbitol from Leaves to Fruit on 'McIntosh' Apples over 24 Hours (1995)

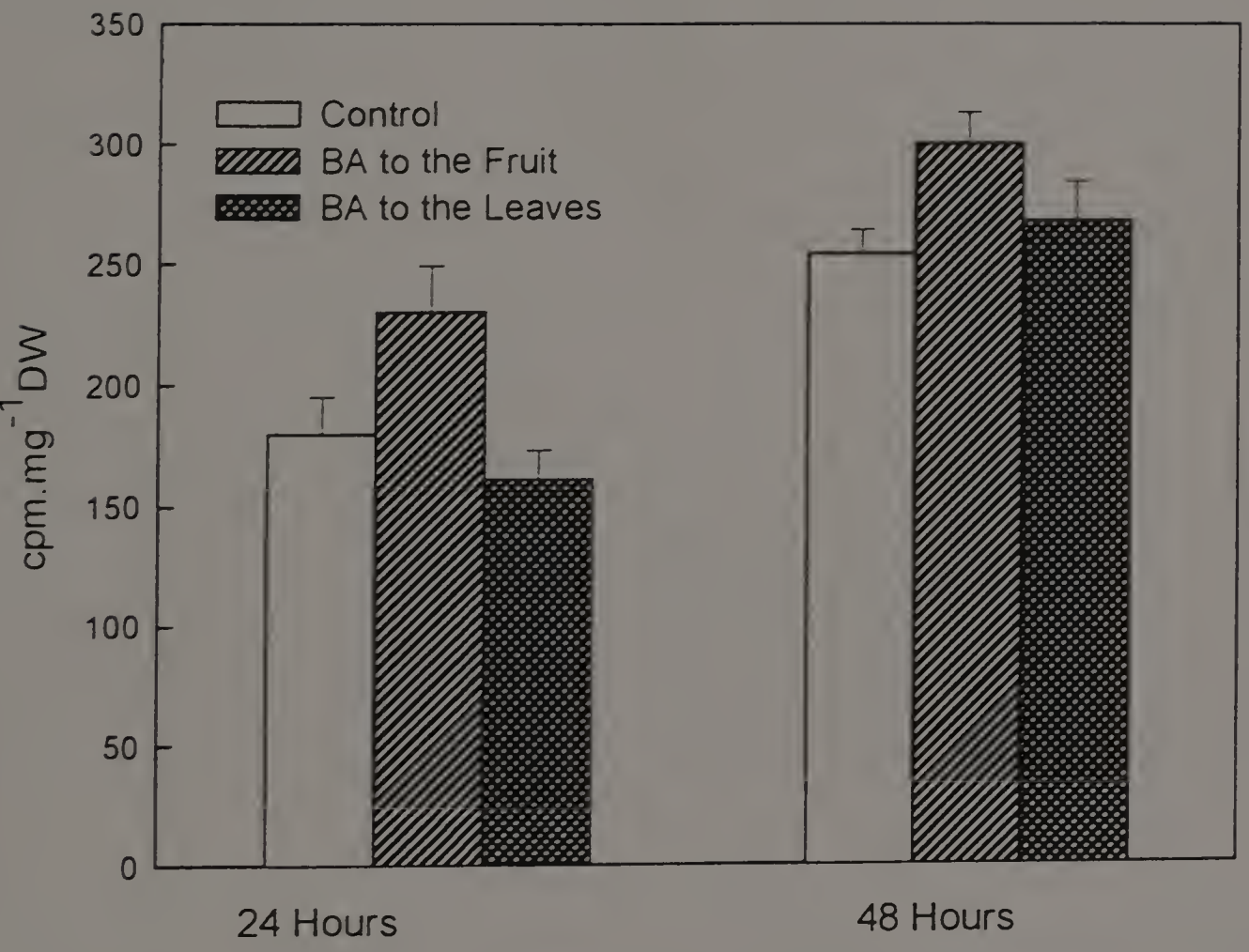

Figure 2. Effects of BA Applied to Leaves or Fruit on the Translocation of ^{14}C-Sorbitol From Leaves to Fruit on 'McIntosh' Apples (1996)

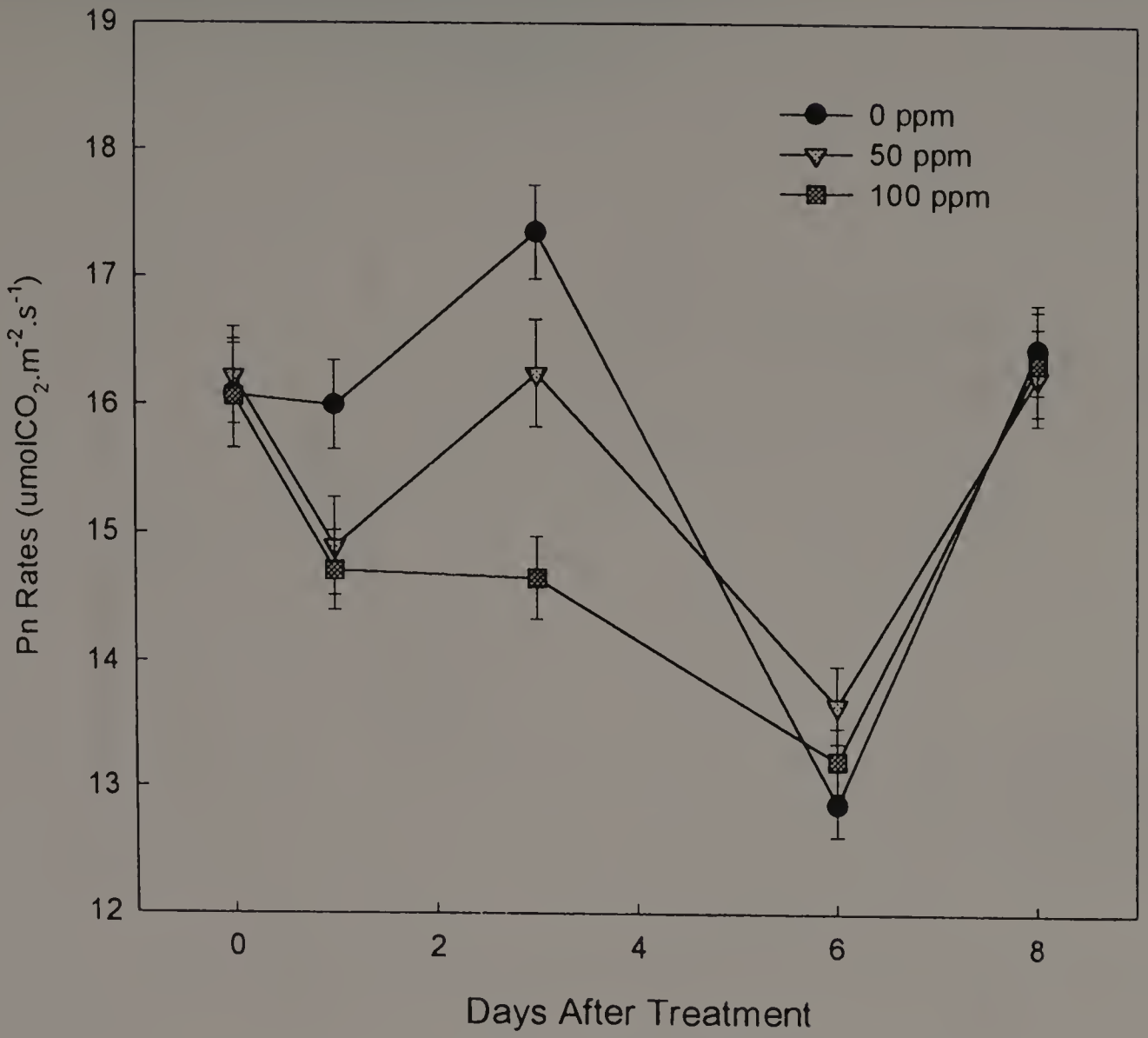

Figure 3. Effects of BA on the Photosynthesis Rates of 'McIntosh' Apple Leaves (1995)

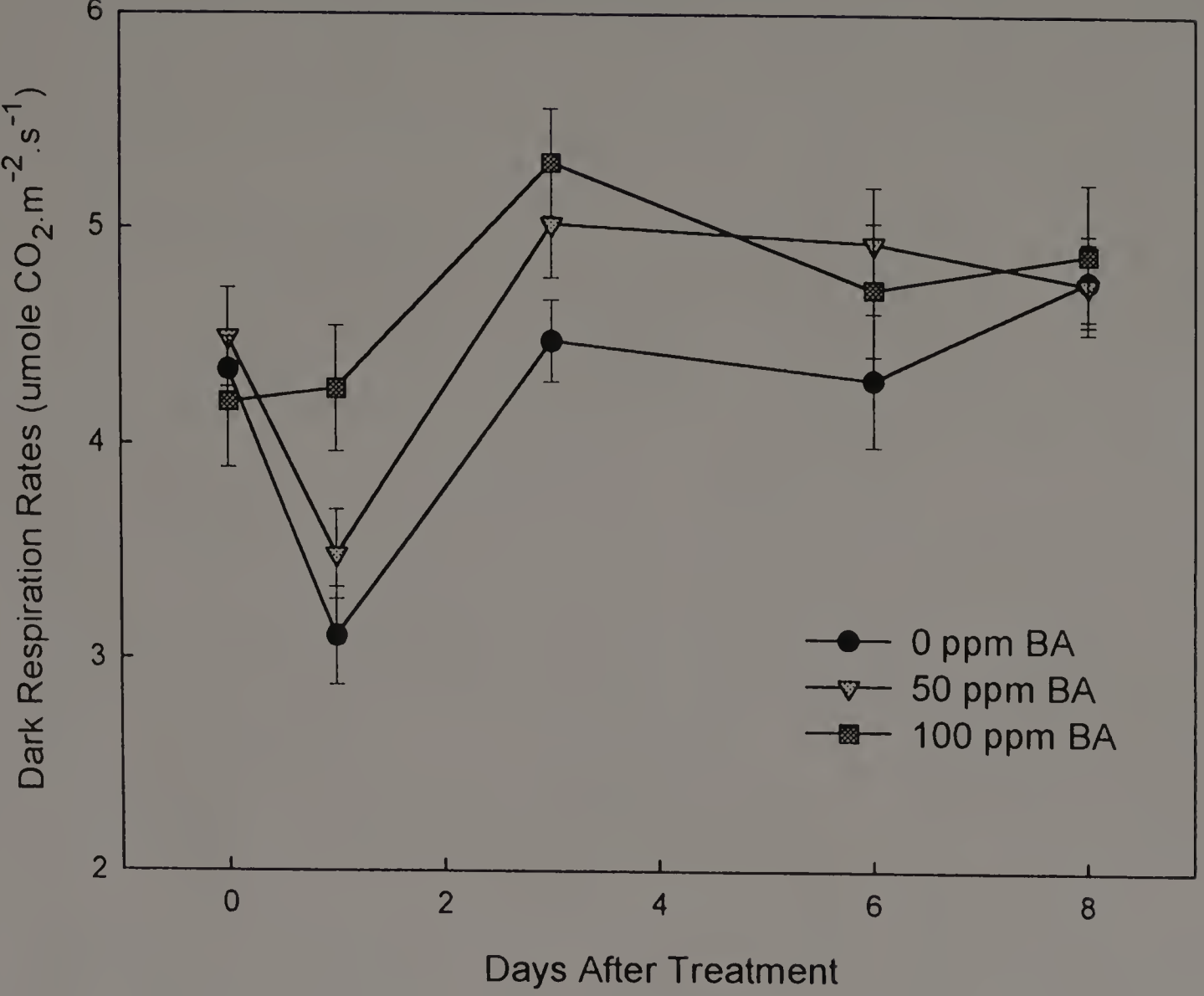

Figure 4. Effects of BA on the Dark Respiration Rates of 'McIntosh' Apple Leaves (1995)

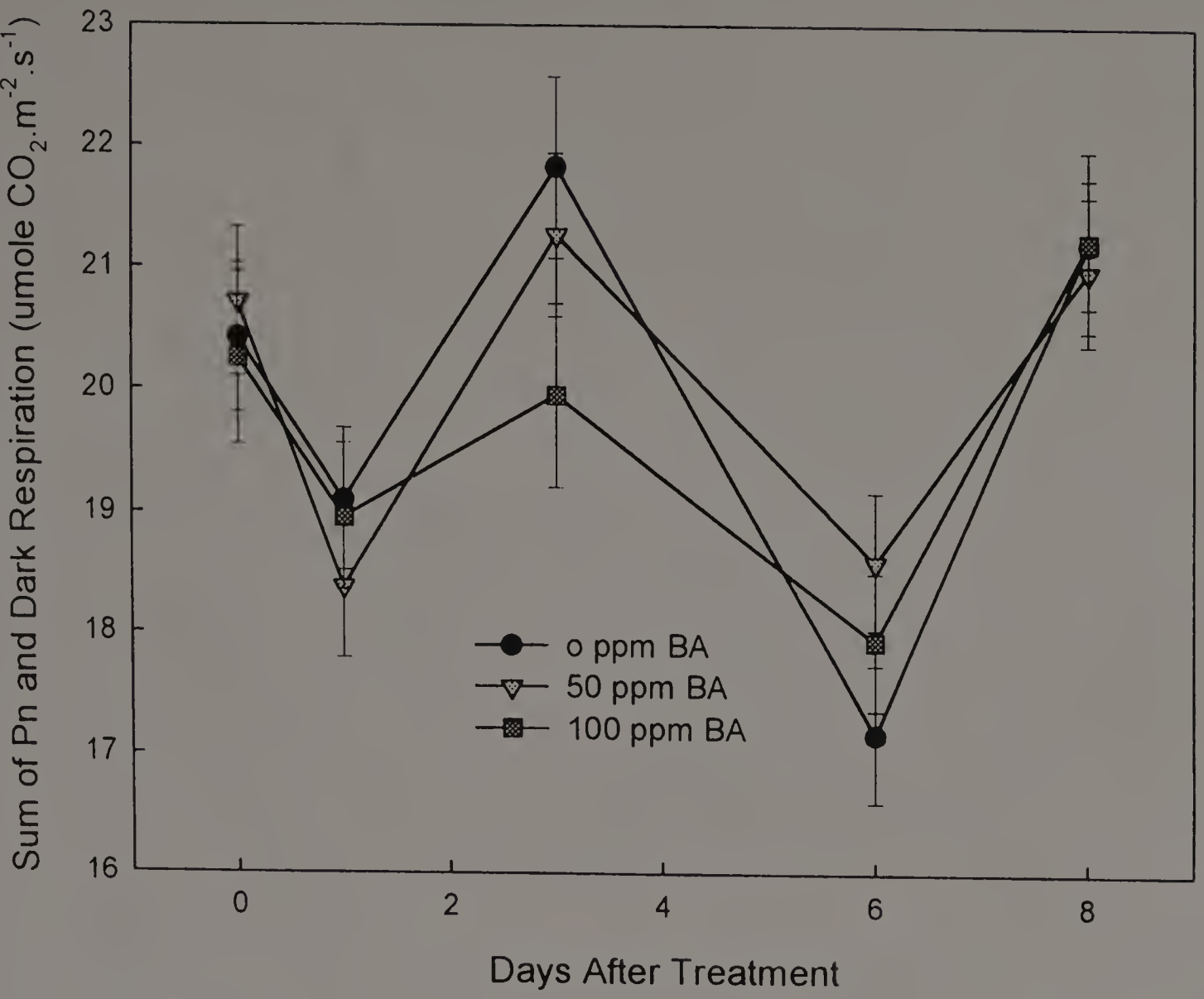

Figure 5. Effects of BA on the Sum of Photosynthesis and Dark Respiration of 'McIntosh' Apple Leaves (1995)

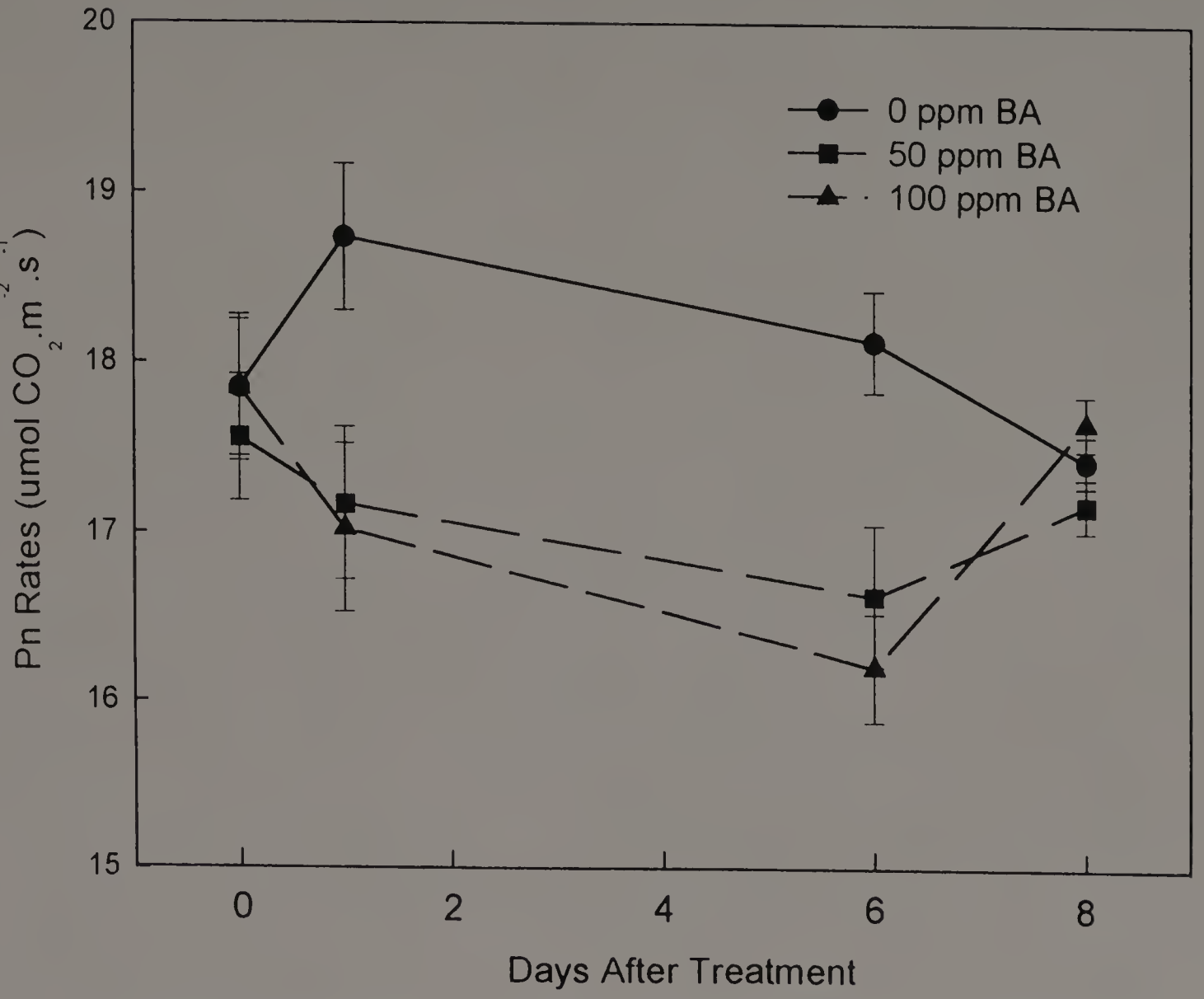

Figure 6 Effects of BA on the Photosynthesis Rates of 'McIntosh' Apple Leaves (1996)

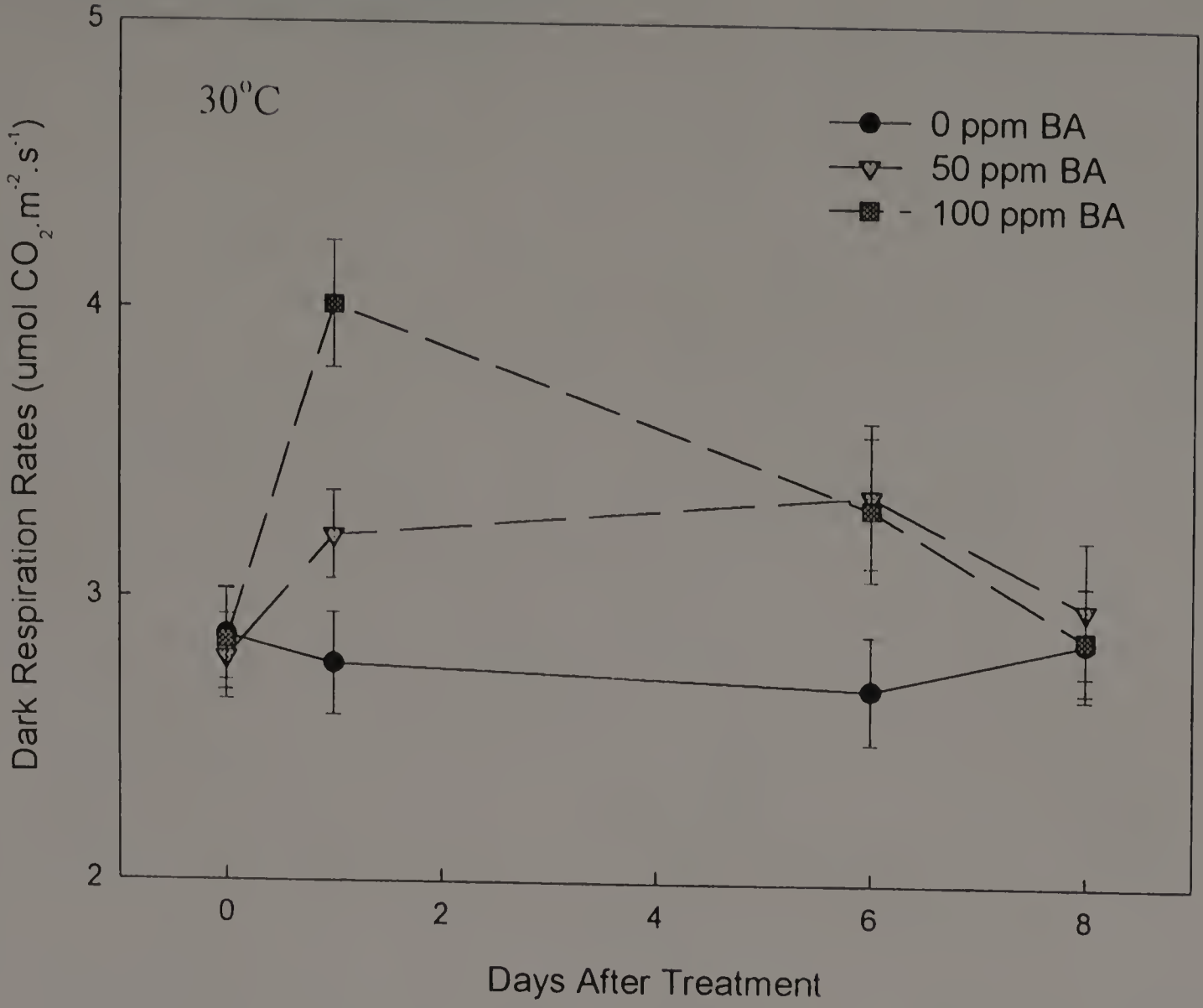

Figure 7 Effects of BA on the Dark Respiration Rates of 'McIntosh' Apple Leaves at about 30°C (1996)

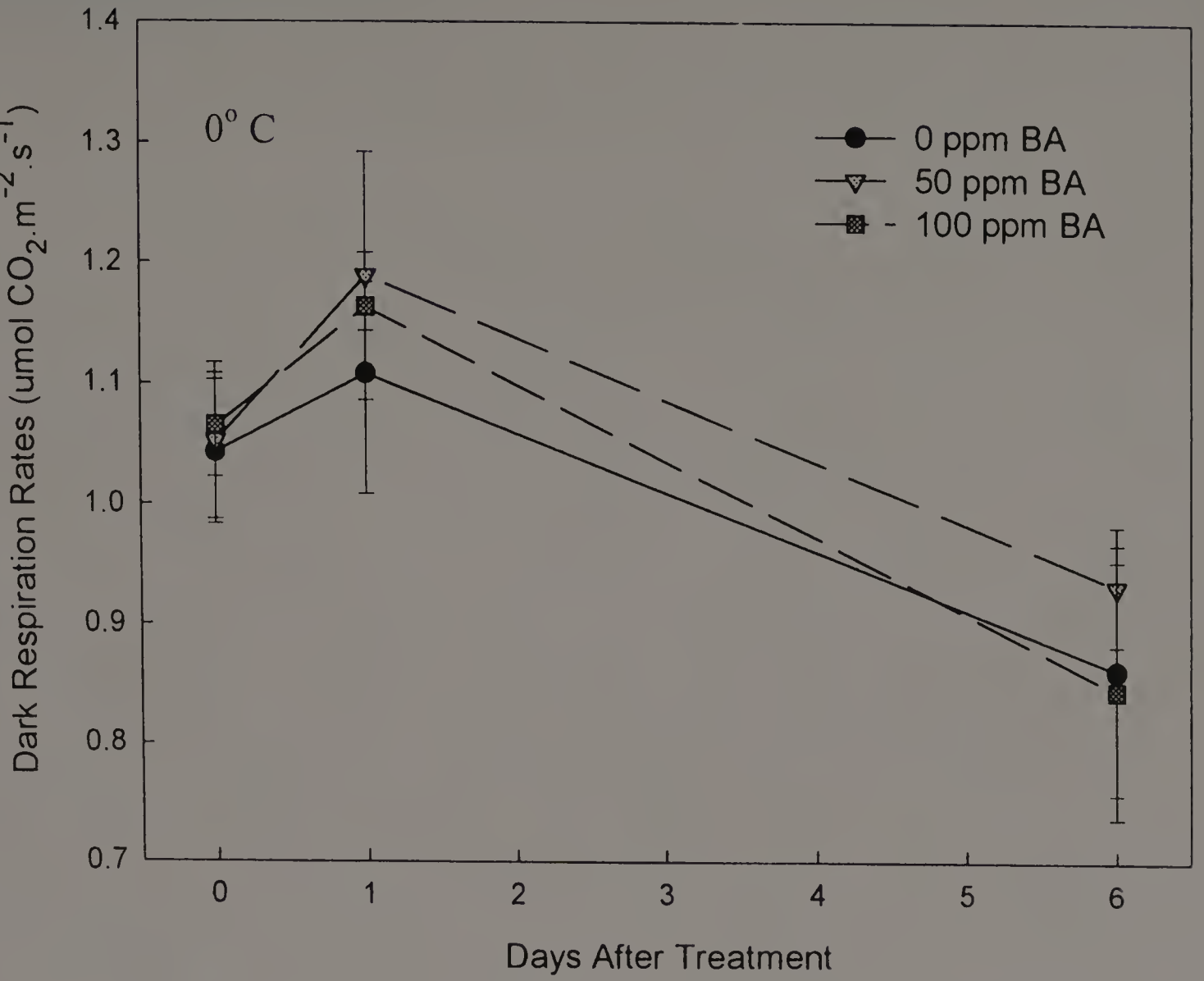

Figure 8. Effects of BA on the Dark Respiration Rates of 'McIntosh' Apples at about 20°C (1996)

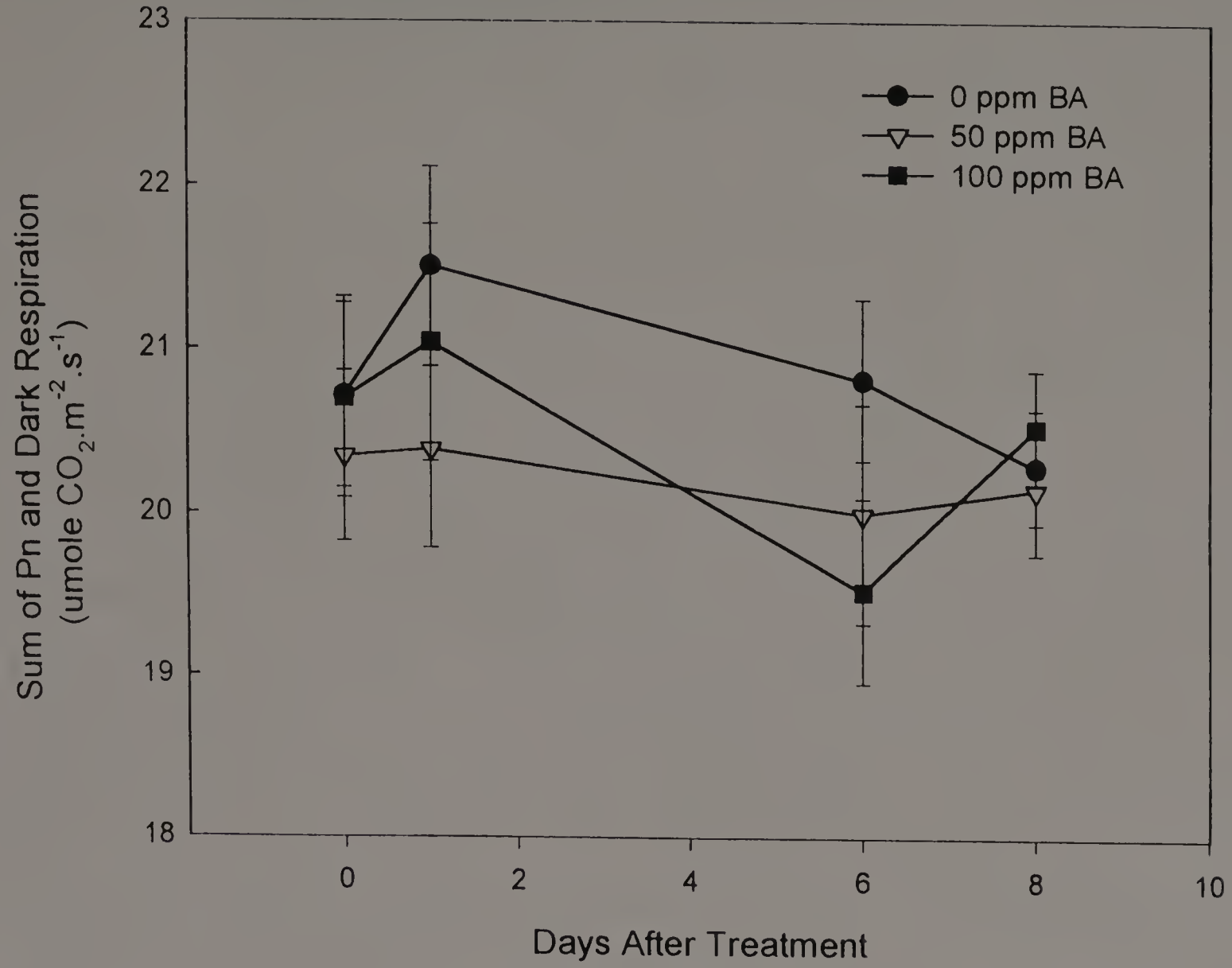

Figure 9. Effects of BA on the Sum of Photosynthesis and Dark Respiration of 'McIntosh' Apple Leaves (1996)

Table 1. Effects of BA on fruit set and fruit characteristics at harvest of 'McIntosh' apple in 1995[z]

BA[y] (ppm)	Blossom cluster/ cm^2 limb X-sect. area	Fruit/ cm^2 limb X-sect. area[x]	Fruit Wt (g)	Flesh firmness	Soluble solids conen (%)	Red color (%)	Normal seeds (no.)	Aborted seeds (no.)	Total seeds (no.)
0	10.3	13.2	124.6	10.6	11.1	74.0	9.5	0.8	10.3
50	10.5	9.8	152.2	10.7	11.3	67.0	10.0	1.3	11.3
100	10.2	7.8	161.1	10.9	11.3	64.0	9.8	1.4	11.2
Significance									
L	NS	**	***	NS	NS	*	NS	**	NS
Q	NS	NS	**	NS	NS	NS	NS	NS	NS

[z]Mean of 8 observations.
[y]BA was applied on June 1, 1995, at 10 mm stage of fruit development.
[x]Fruit set on July 16, 1995.
***,**,*,[NS]Significant at P=0.001, 0.01, 0.05 or nonsignificant, respectively.

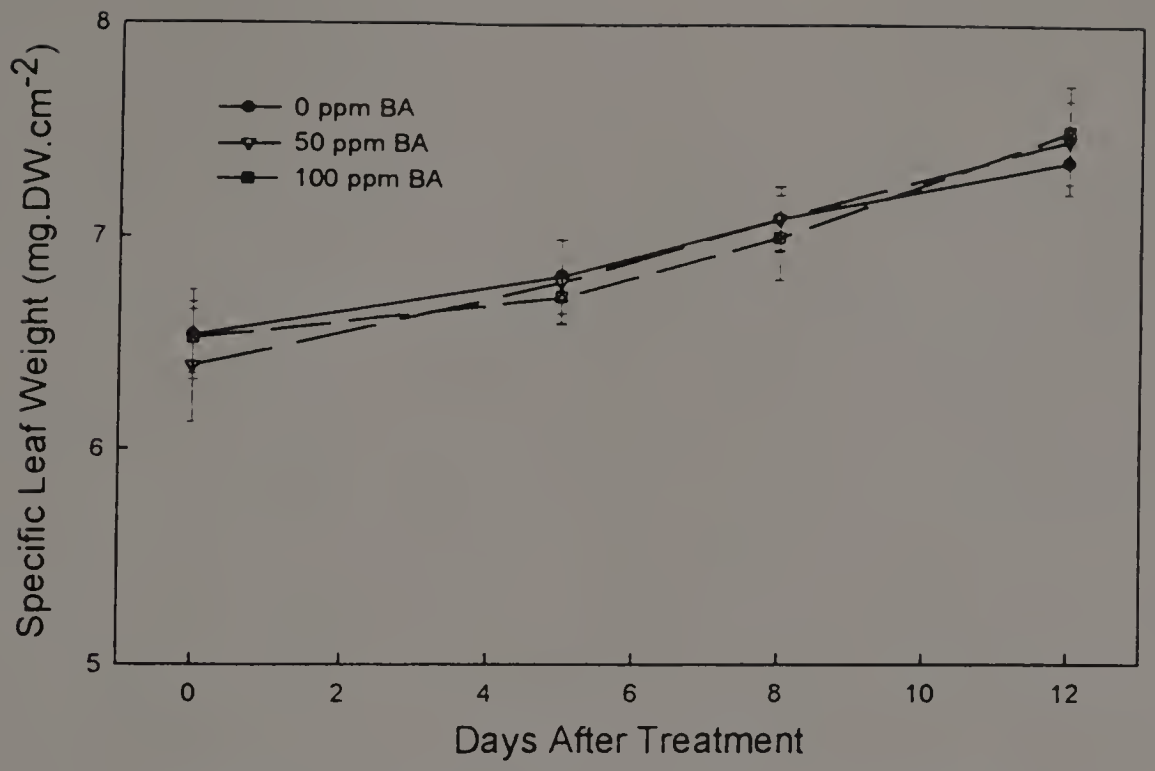

Figure 10. Effects of BA on Specific Leaf Weight of 'McIntosh' Apples (1995)

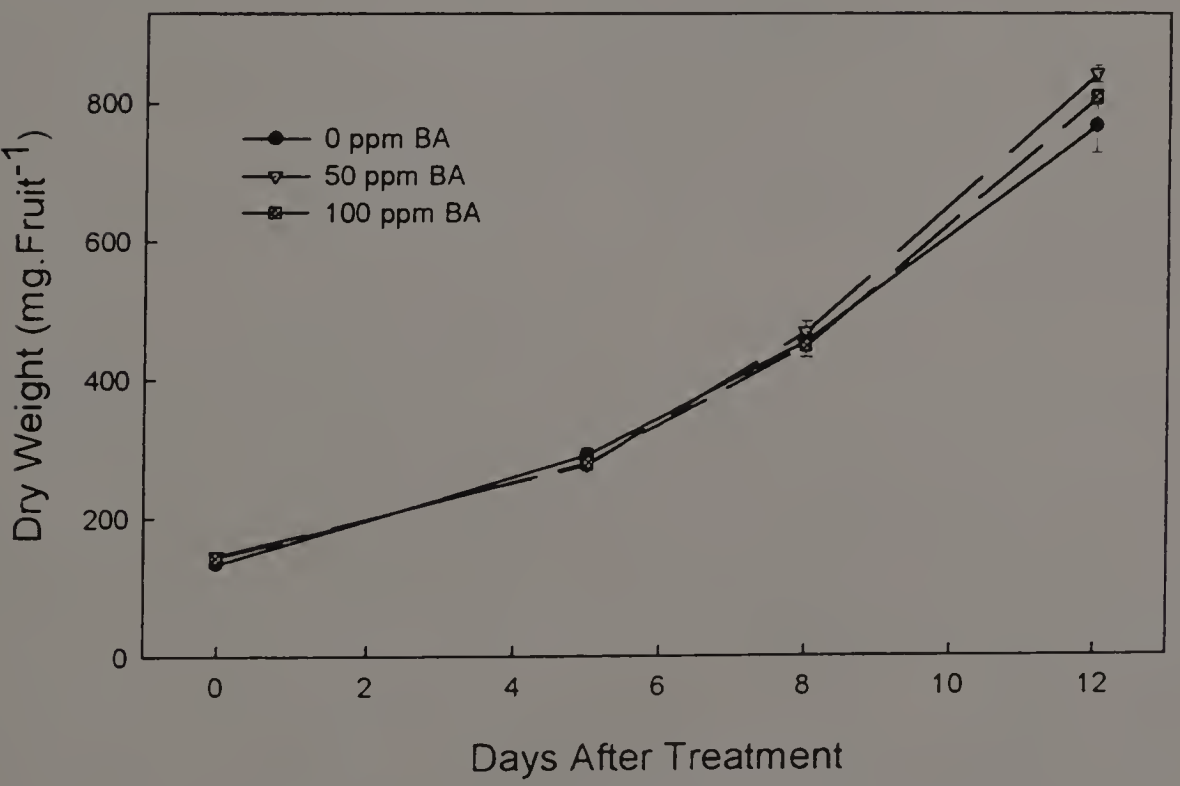

Figure 11 Effects of BA on Fruit Dry Weight of 'McIntosh' Apples (1995)

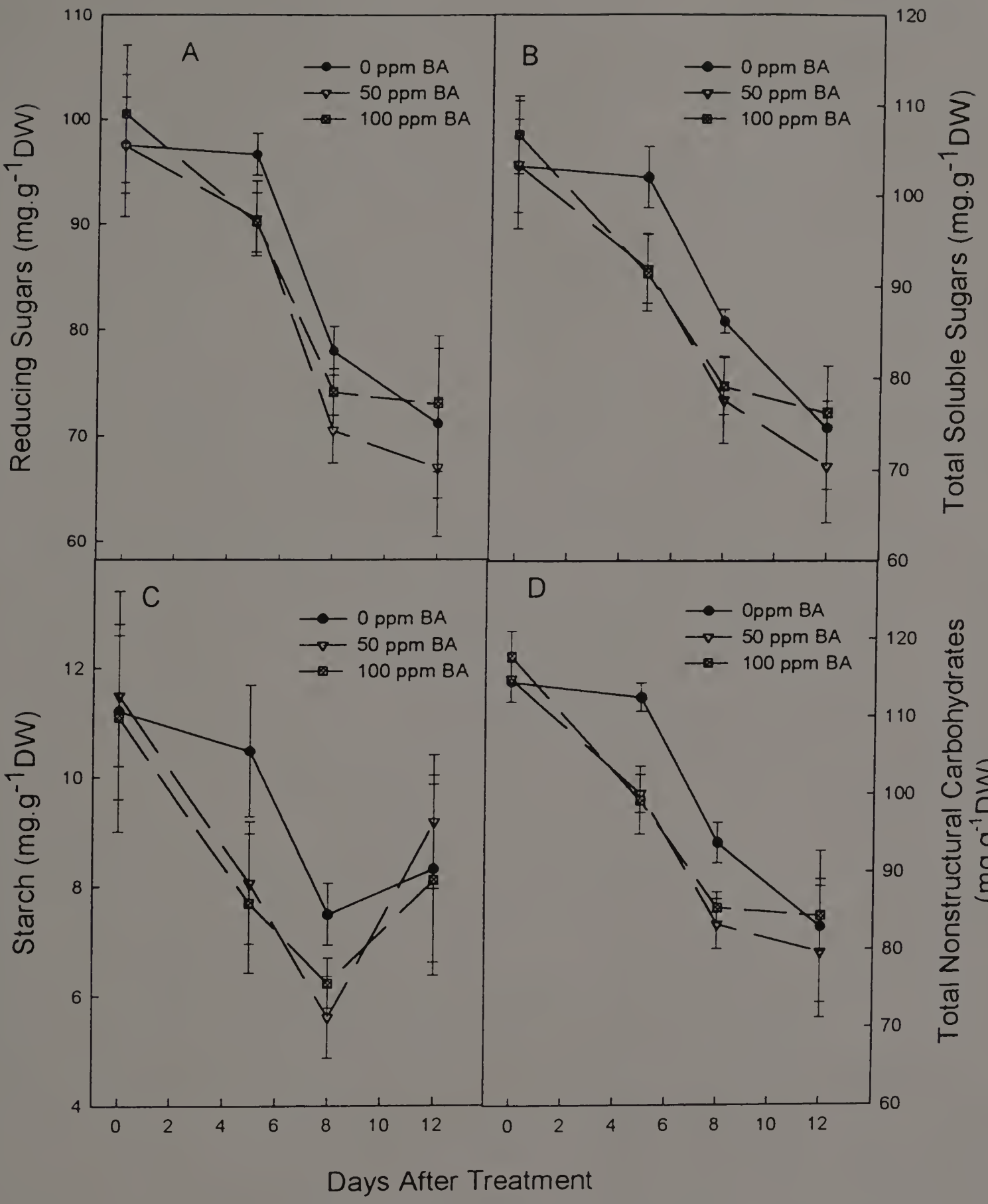

Figure 12. Effects of BA on Nonstructural Carbohydrate Levels of 'McIntosh' Apple Leaves (1995)

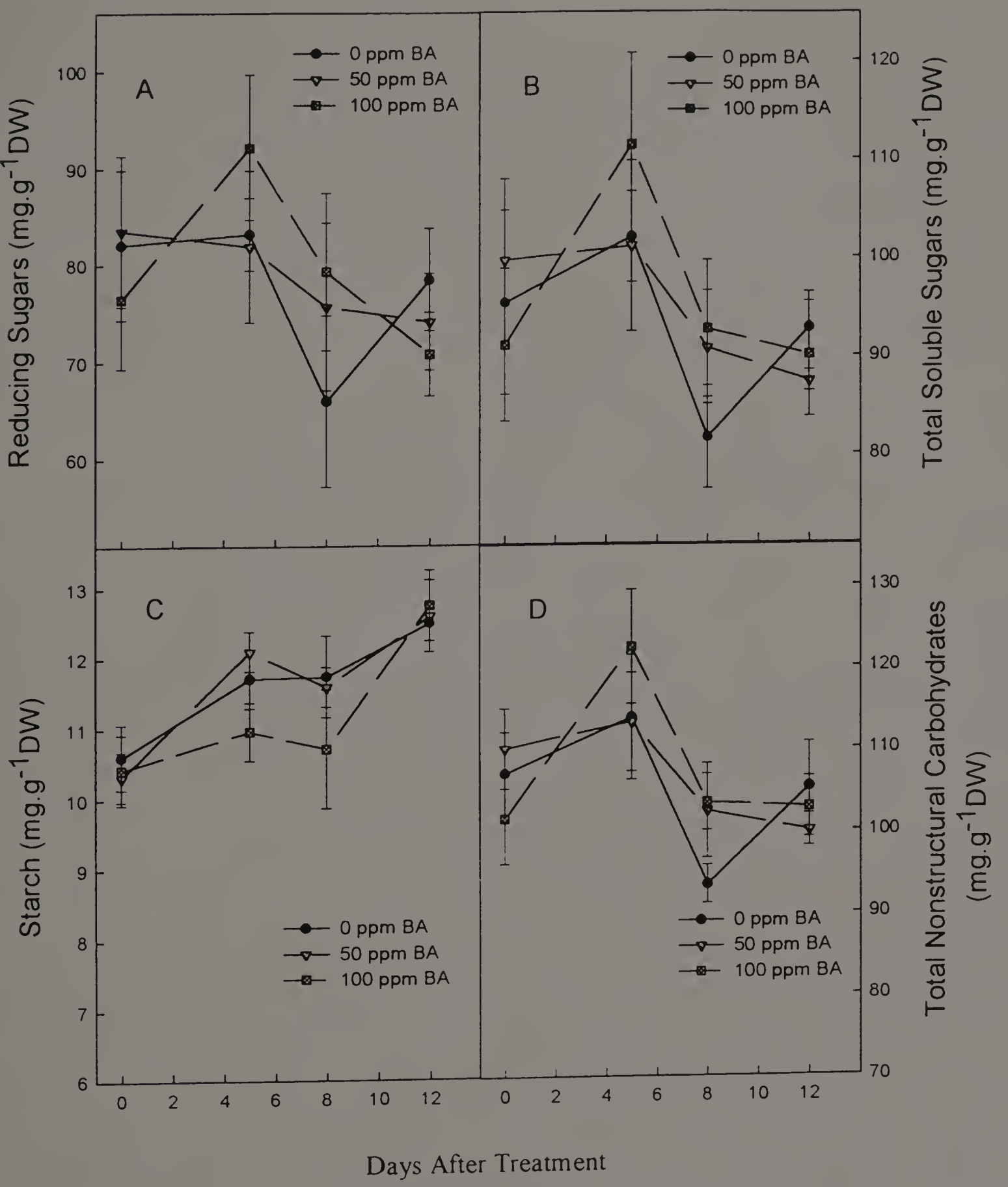

Figure 13. Effects of BA on Nonstructural Carbohydrate Levels of 'McIntosh' Apple Fruit (1995)

Table 2. Effects of BA on fruit set and fruit characteristics at harvest of 'McIntosh' apple in 1996[z]

BA[y] (ppm)	Blossom cluster/ cm^2 limb X--sect. area	Fruit/ cm^2 limb X-sect. area[x]	Fruit Wt (g)	Flesh firmness	Soluble solids concn (%)	Red color (%)	Normal seed (no.)	Aborted seed (no.)	Total seed (no.)
0	7.1 6.3		156.3	15.5	12.5	50.0	8.0	2.3	10.3
50	7.0 3.9		163.3	15.4	12.3	44.0	8.0	3.4	11.4
100	6.8 3.0		181.6	15.3	12.5	41.0	7.1	3.8	11.0
Significance									
L	NS	**	***	NS	NS	*	NS	*	NS
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Mean of 8 observations.
[y]BA was applied on May 30, 1996, at 10 mm stage of fruit development.
[x]Fruit set on July 17, 1996.
***,**,*,[NS]Significant at P=0.001, 0.01, 0.05 or nonsignificant, respectively.

Table 3. Effects of BA on seed count of abscising and persisting fruit of apples during 'June Drop'(1996)[z]

BA[y] (mg.liter^{-1})	Abscising Total seeds	Persisting Normal seeds	Persisting Aborted seeds	Persisting Total seeds
	(no.)	(no.)	(no.)	(no.)
0	7.1	9.5	0.4	9.9
50	7.7	8.6	1.1	9.6
100	8.0	8.2	1.6	9.7
Significance				
Fruit type[x]	7.6 b		9.7 a	
BA				
L	**	*	**	NS
Q	NS	NS	NS	NS

[z]Mean of 7 observations.

[y]BA was applied on May 30, 1996, at 10 mm stage of fruit development.

[x]Seed number of abscising and persisting fruit was significantly different by F test at P=0.05.

**, *, NS Significant at P=0.01, 0.05 or nonsignificant, respectively.

Table 4. Effects of BA on fruit set and fruit weight in 1997[z]

BA[y] (ppm)	Blossom cluster/ cm^2 limb X--sect. area	Fruit/ cm^2 limb X-sect. area[x]	Fruit Wt (g)
0	14.4	11.3	110.9
100	14.7	5.5	170.8
Significance	NS	**	***

[z]Mean of 4 observations.

[y]BA was applied on June 6, 1997, at 10 mm stage of fruit development.

[x] Fruit set on July 11, 1997.

***,**,*,[NS]Significant at P=0.001, 0.01, 0.05 or nonsignificant, respectively.

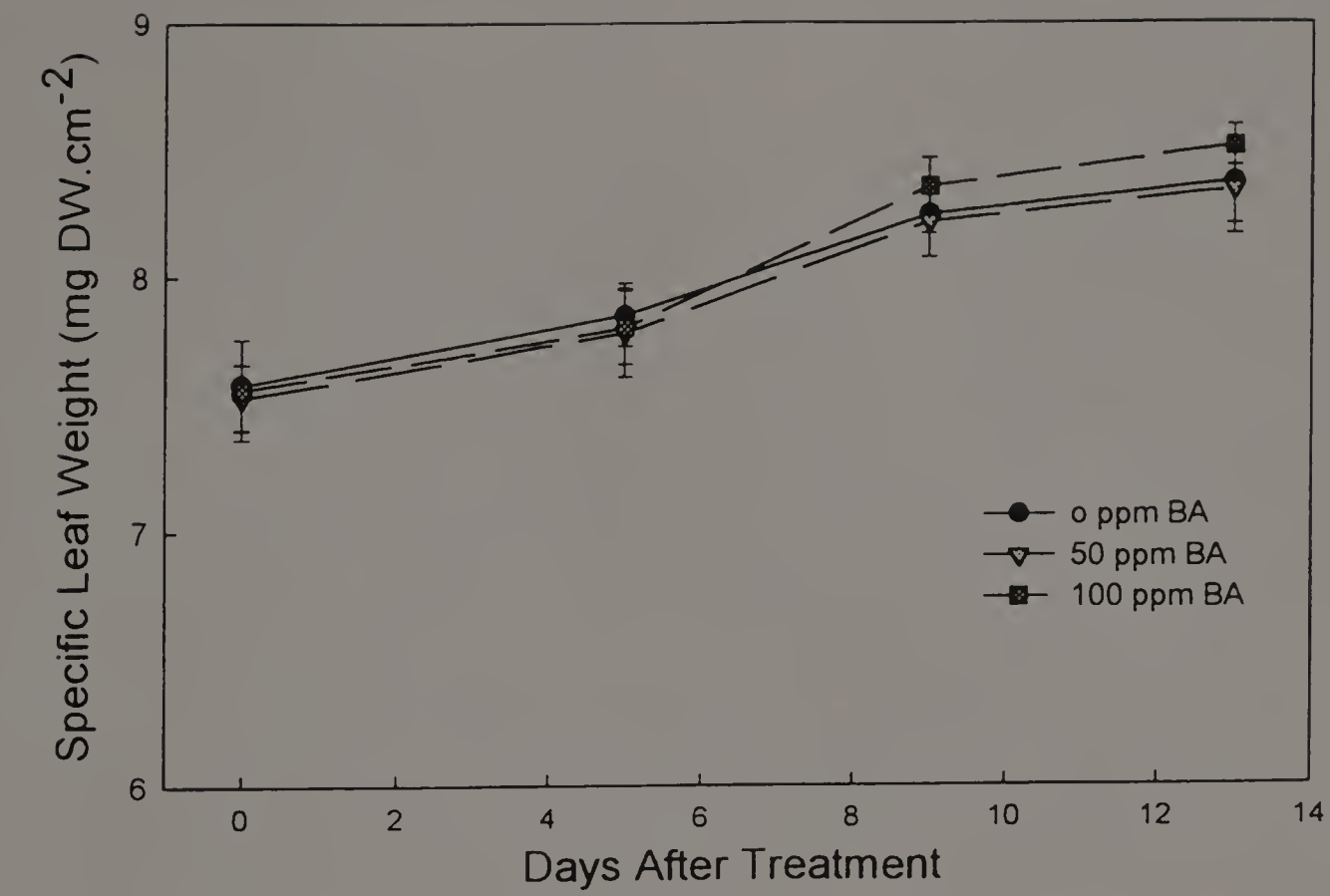

Figure 14. Effects of BA on Specific Leaf Weight of 'McIntosh' Apples (1996)

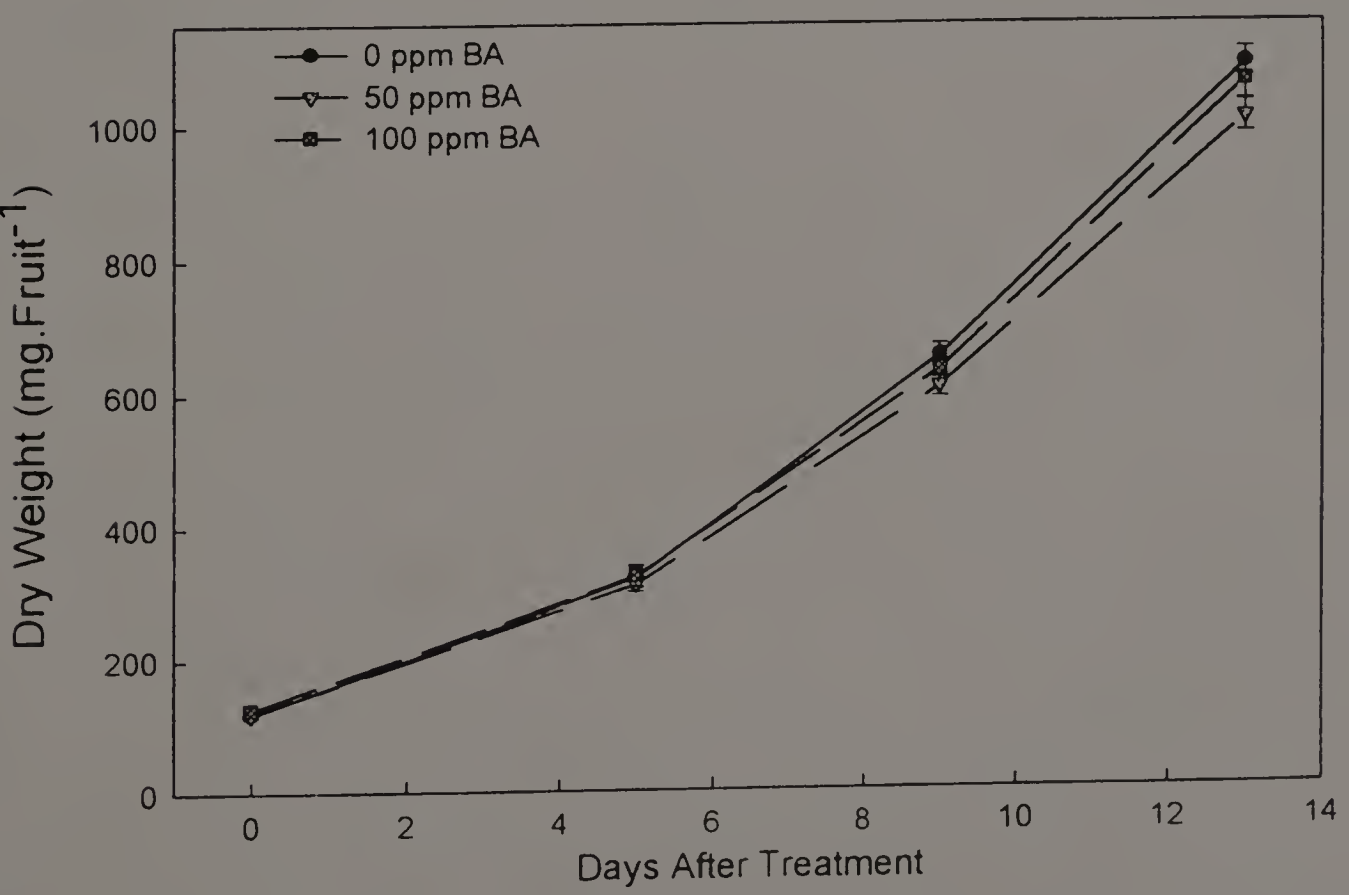

Figure 15 Effects of BA on Fruit Dry Weight of 'McIntosh' Apples (1996)

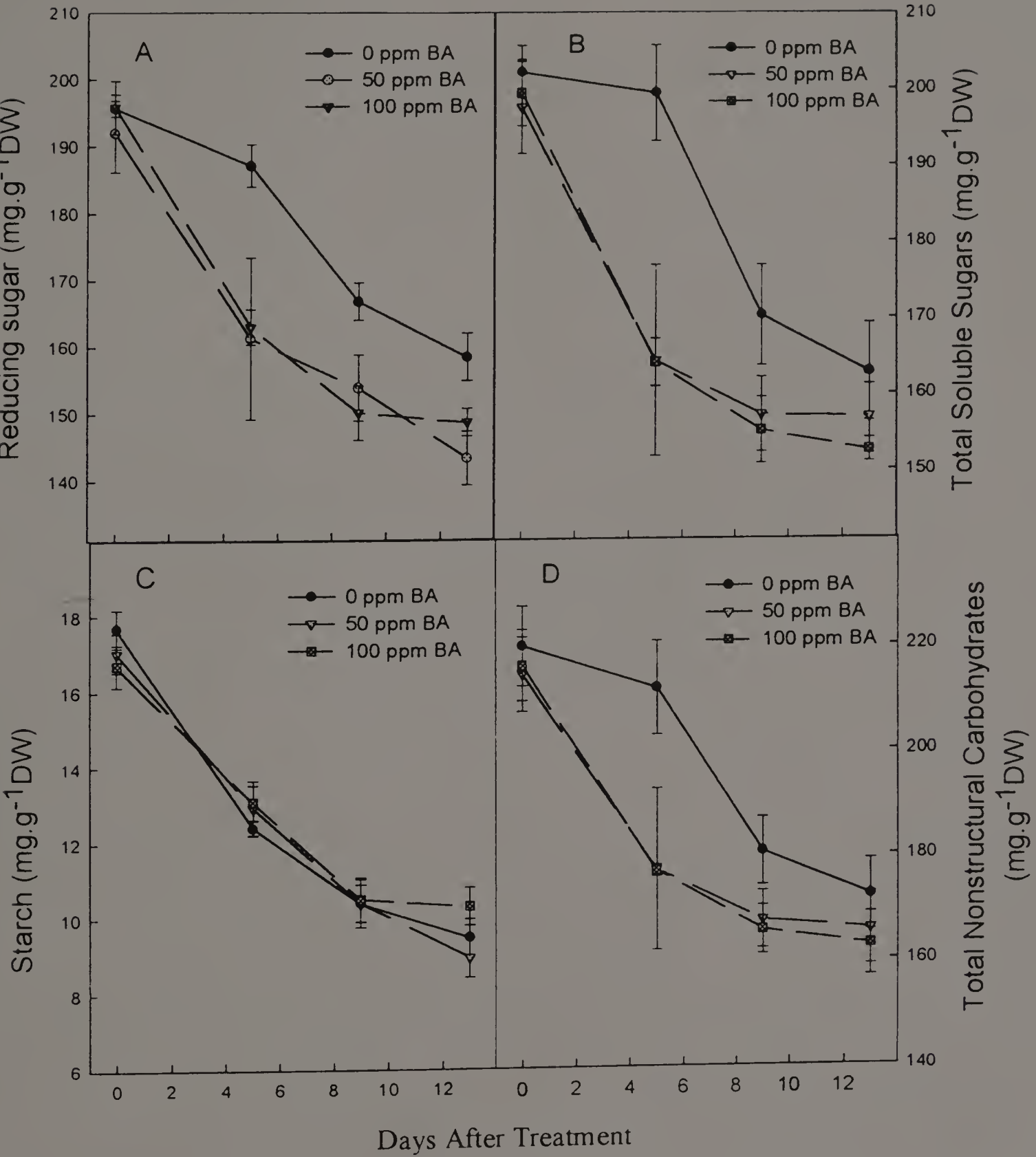

Figure 16. Effects of BA on Nonstructural Carbohydrate Levels of 'McIntosh' Apple Leaves (1996)

Table 5. The effects of BA on fruit fresh weight, dry weight and fruit size of persisting and abscising fruit (1997)[z]

BA[y] (ppm)	Persisting (June 18)[u]	Abscising (June 18)[u] (same age)	Abscising (June 30)[v] (same size)
		Fruit Size (mm)	
0	25.4	17.58	25.21
100	25.76[ns]	17.21[ns]	25.58[ns]
Mean	25.58 a[x]	17.39 b	25.39 a
		F.W.Fruit^{-1}(g)	
0	8.16	2.83	7.42
100	8.28[ns]	2.86[ns]	7.79[ns]
Mean	8.22 a	2.85 c	7.60 b
		D.W.Fruit^{-1} (g)	
0	0.85	0.37	0.93
100	0.93[ns]	0.36[ns]	0.98[ns]
Means	0.89 b	0.365 c	0.955 a
		Water.Fruit^{-1} (g)	
0	7.31	2.46	6.49
100	7.35[ns]	2.5[ns]	6.81[ns]
Mean	7.33 a	2.48 c	6.65 b

[z]Mean of 4 observations.

[y]BA was applied, on June 6,1997, at 10 mm stage of fruit development.

[u] and [v] 12 and 24 days after BA application.

[x]Mean separation within row by Duncan's new multiple range test (P=0.05).
[ns]The difference between 0 and 100 ppm BA was nonsignificant.

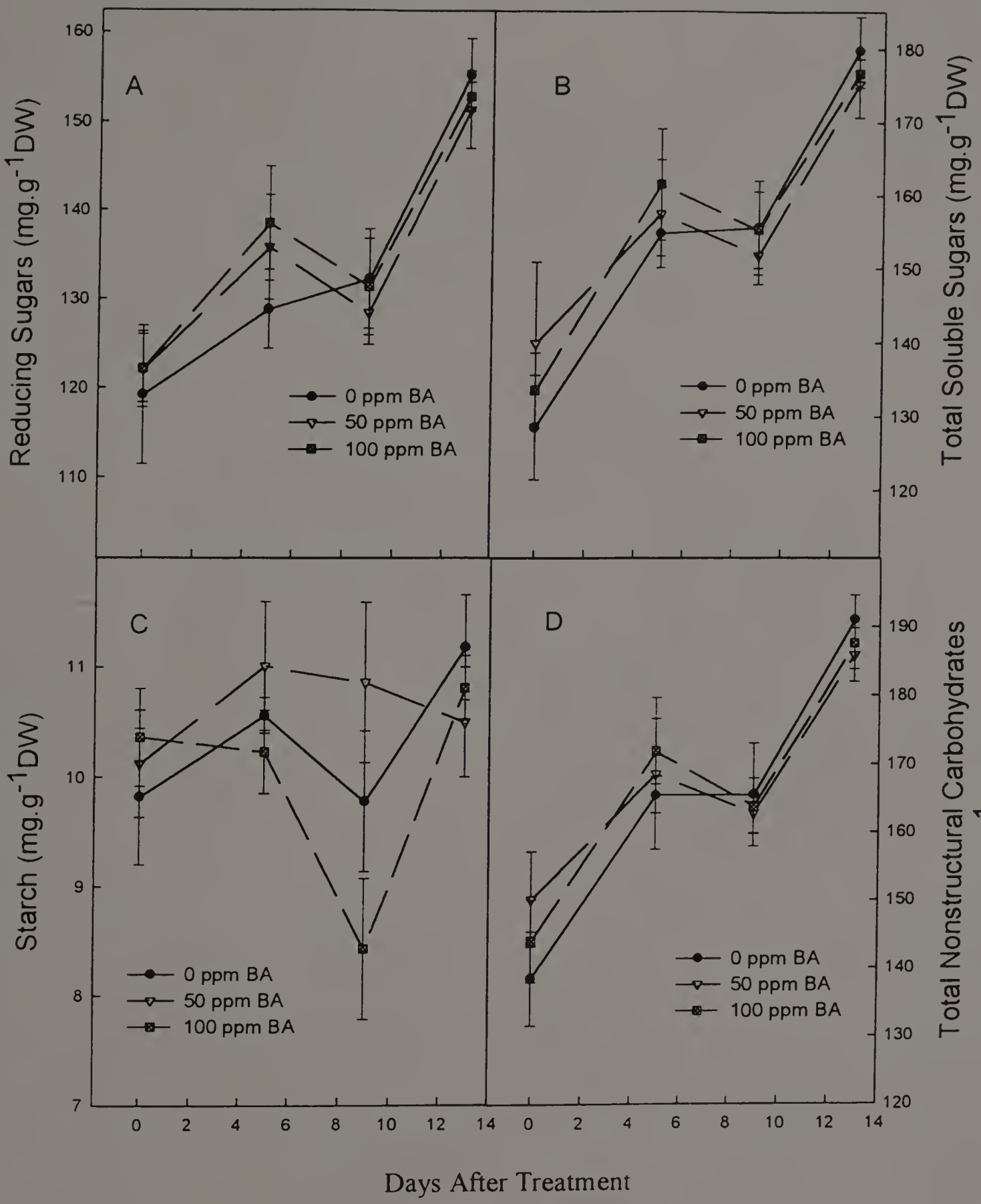

Figure 17. Effects of BA on Nonstructural Carbohydrate Levels of 'McIntosh' Apple Fruits (1996)

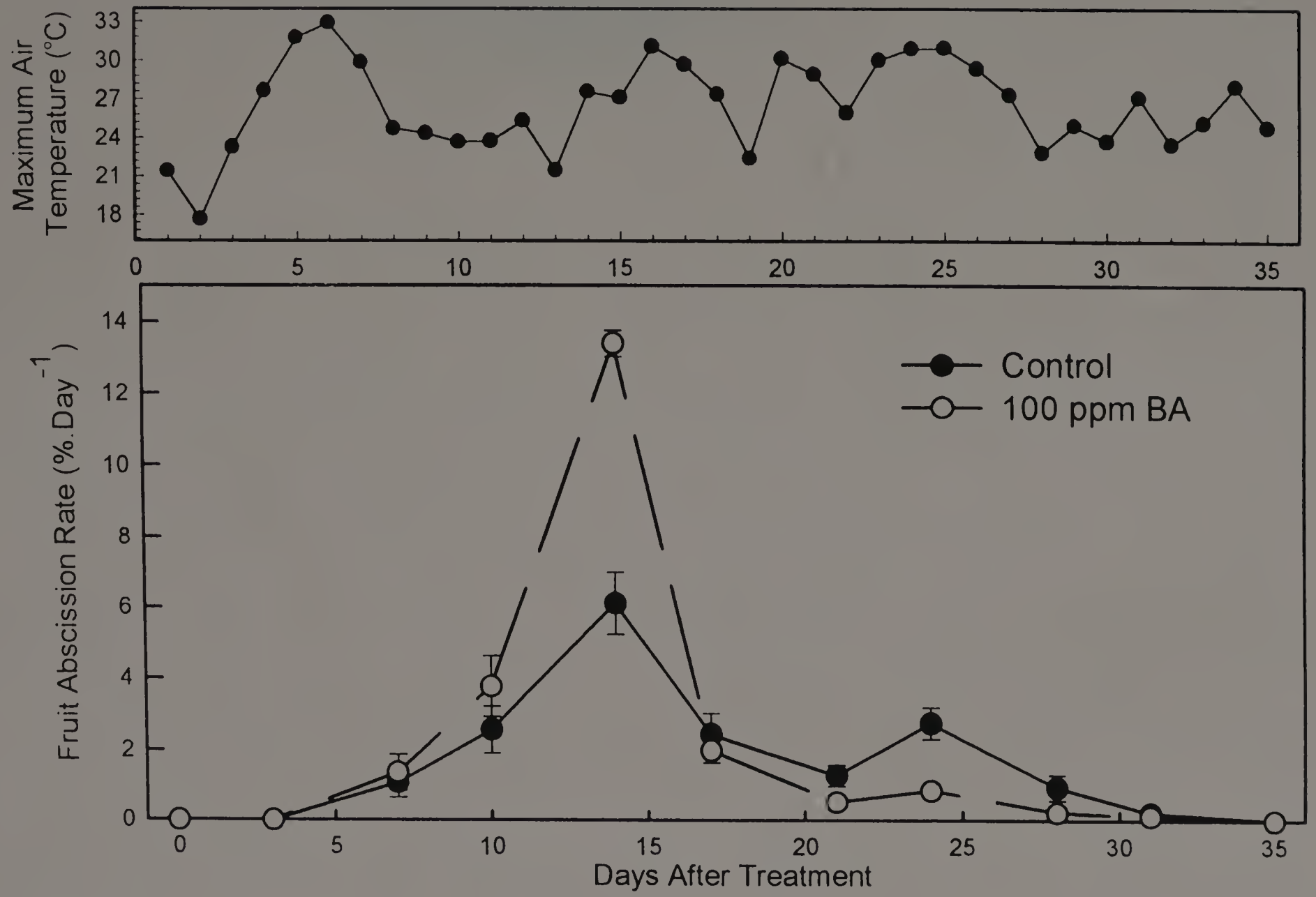

Figure 18. Effects of BA on the Patterns of Fruit Abscission of 'McIntosh' Apples During 'June Drop' as Related to Maximum Air Temperature (1997)

Table 6. Effects of BA on reducing sugars, total soluble sugars, starch and total nonstructural carbohydrates of persisting and abscising fruits (1997)[z]

BA[y] (ppm)	Persisting (June 18)[u]	Abscising (June 18)[u] (same age)	Abscising (June 30)[v] (same size)
		Reducing Sugar	
0	185.88	219.09	294.94
100	181.65[ns]	229.66[ns]	303.77[ns]
Means	183.76 c[x]	224.37 b	299.36 a
		Total soluble sugars	
0	198.37	228.20	312.66
100	194.57[ns]	238.27[ns]	320.02[ns]
Means	196.20 c	233.24 b	316.34 a
		Starch	
0	18.81	15.65	27.52
100	15.00[ns]	13.18[ns]	26.32[ns]
Means	16.91 b	14.42 b	26.92 a
		TNC	
0	214.94	243.85	340.18
100	210.07[ns]	251.46[ns]	346.34[ns]
Means	212.16 c	247.65 b	343.26 a

[z]Mean of 5 observations.

[y]BA was applied,on June 6,1997, at 10 mm stage of fruit development.

[u] and [v] 12 and 24 days after BA application.

[x]Mean separation within row by Duncan's new multiple range test (P=0.05).

[ns]The difference between 0 and 100 ppm BA was nonsignificant.

Table 7. Effects of BA and sorbitol on fruit set and fruit quality at harvest of 'McIntosh' apples (1995)[z]

Treatments		Fruit/cm² limb cross-sec. area			Fruit Wt	Red color	Seed number			Flesh	Soluble solids
Sorbitol[y]	BA[y]	June 1	June 16	July 8	(g)	(%)	Normal	Aborted	Total	firmness	concn (%)
–	-	25.7	17.9	14.9	118.4	73.0	10.0	1.8	11.8	9.8	10.8
+	-	22.1	17.9	13.8	122.6	67.0	9.8	1.7	11.5	10.2	11.2
+	+	22.9	11.5	8.6	152.0	74.0	9.5	2.2	11.7	10.1	11.7
-	+	22.1	10.9	8.4	143.4	62.0	10.3	1.9	12.2	10.2	11.4
Significance											
BA		NS	***	***	***	NS	NS	*	NS	NS	**
Sorbitol		NS	NS	NS	**	NS	NS	NS	NS	NS	NS
BA×Sorbitol		NS	NS	NS	NS	NS	*	NS	NS	NS	NS
Sorbitol:BA-		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sorbitol:BA+		NS	NS	NS	NS	NS	*	NS	NS	NS	NS

[z]Means of 8 observations.

[y]Treatments were applied on June 1, 1995, at 10 mm stage of fruit development.

[**,*,NS]Significant at P=0.01, 0.05 or nonsignificant, respectively.

Table 8. Effects of BA and bourse shoot tip removal on fruit set and fruit quality at harvest of 'McIntosh' apples (1996)[z]

Treatments		Fruits/cm limb cross-sec. area		Fruit Wt	Red color	Seed number			Flesh firmness	Soluble solids
Shoots[y]	BA[y]	May 30	July 8	(g)	(%)	Normal	Aborted	Total		concn (%)
+[x]	-	17.1	6.2	159.6	45.0	7.6	2.1	9.7	14.9	11.6
-	-	16.8	6.9	150.2	40.0	7.5	1.8	9.3	15.2	11.6
+	+	18.0	2.4	188.0	29.0	5.7	4.2	9.9	15.1	11.9
-	+	19.1	3.8	183.6	31.0	7.5	2.2	9.7	14.9	11.6
Significance										
BA		NS	**	**	**	**	*	NS	NS	NS
Shoot		NS	*	NS	NS	*	NS	NS	NS	NS
BA× Shoot		NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Means of 8 observations.

[y]BA and tipping were applied, on May 30,1996, at 10 mm stage of fruit development.

**,*,[NS]Significant atP= 0.01, 0.05 or nonsignificant, respectively.

[x]With the presence of shoot tip.

Table 9. Effects of BA and different leaf number per fruit on fruit set and quality at harvest of 'McIntosh' apples (1996)[z]

BA[y] (ppm)	Leaf number[y]	Fruit		Fruit abscission rate(%)	Fruit weight (g)	Flesh Firmness	Soluble solids content(%)	Seed number		
		Initial	Final					Viable	Aborted	Total
0	25	1.0	1.0	0	135.4	17.7	12.3	8.3	2.3	10.6
	50	1.0	1.0	0	151.3	18.0	11.7	8.7	2.0	10.7
100	25	1.0	1.0	0	154.2	18.2	11.6	9.1	2.4	11.5
	50	1.0	1.0	0	170.5	17.5	12.5	8.9	2.6	11.5
Significance										
BA		NS	NS	NS	**	NS	NS	NS	NS	NS
Leaves		NS	NS	NS	**	NS	NS	NS	NS	NS
BA×Leaves		NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Mean of 15 observations.

[y]Treatments were applied on May 30, 1996, at 10 mm stage of fruit development.

**, [NS]Significant at P=1% or not significant, respectively.

Table 10. Effects of BA and different leaf number per fruit on fruit set of 'McIntosh' apples (1997).[z]

BA[y] (ppm)	Leaf number[y]	Leaf area (cm^2)	Fruit		
			Fruit		abscission
			Initial	Final	rate(%)
0	1	31.5	1.0	0.75	25.0
	2	56.8	1.0	1.0	0.0
	4	112.8	1.0	1.0	0.0
	8	210.0	1.0	1.0	0.0
	16	412.4	1.0	1.0	0.0
	32	874.9	1.0	1.0	0.0
100	1	31.5	1.0	0.125	87.5
	2	57.1	1.0	1.0	0.0
	4	121.1	1.0	1.0	0.0
	8	208.5	1.0	1.0	0.0
	16	412.1	1.0	1.0	0.0
	32	886.2	1.0	1.0	0.0
Significance					
BA		NS			***
Leaves		L***Q***			***
BA×Leaves					***
	BA:Leaves 1				***
	BA:Leaves 2				NS
	BA:Leaves 4				NS

[z]Mean of 16 observations.

[y]Treatments were applied on June 6, 1997, at 10 mm stage of fruit development.

***, [NS]Significant at P=0.001, or nonsignificant, respectively.

Table 11. Effects of BA and different leaf number on fruit quality at harvest of 'McIntosh' apples (1997)[z]

BA[y] (ppm)	Leaf number[y]	Soluble solids content(%)	Fruit weight (g)	Seed count		
				Normal	Aborted	Total
0	1	8.2	24.2	5.8	6.2	12.0
	2	9.2	58.5	9.7	1.8	11.5
	4	9.6	92.6	11.5	0.8	12.3
	8	10.7	118.1	10.2	1.0	11.2
	16	11.4	130.0	10.2	0.9	11.0
	32	11.9	135.6	9.9	0.9	10.8
100	1	8.8	32.0	1.5	9.0	10.5
	2	9.2	66.7	7.9	3.5	11.4
	4	9.8	97.2	8.8	1.9	10.7
	8	10.6	117.6	9.1	1.2	10.3
	16	11.5	129.1	10.0	0.7	10.7
	32	12.5	147.8	9.9	0.9	10.8
Sig.						
BA		NS	NS	*	NS	NS
Leaves		L***Q^{ns}	L***Q***	L***Q**	L***Q***	NS
BA×Leaves		NS	NS	*	NS	NS
BA:Leaves 1				*		
BA:Leaves 2				*		
BA:Leaves 4				*		
BA:Leaves 8					NS	

[z]Mean of 16 observations.

[y]Treatments were applied on June 6, 1997, at 10 mm stage of fruit development.

***,**,*,^{NS}Significant at P=0.001, 0.01, 0.05 or nonsignificant, respectively.

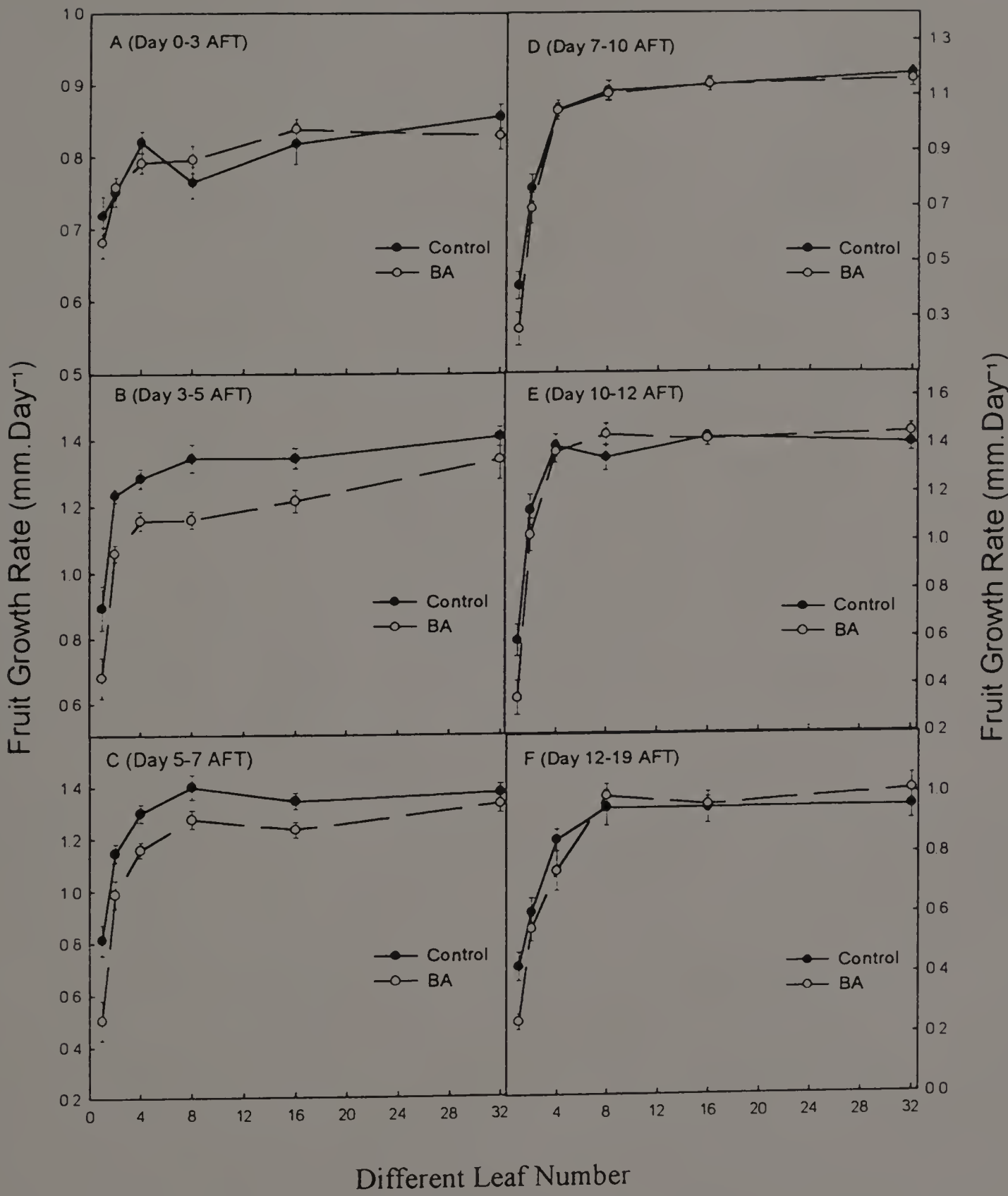

Figure 19. Effects of BA and Different Leaf number Per Fruit on Fruit Growth Rate of 'McIntosh' Apples

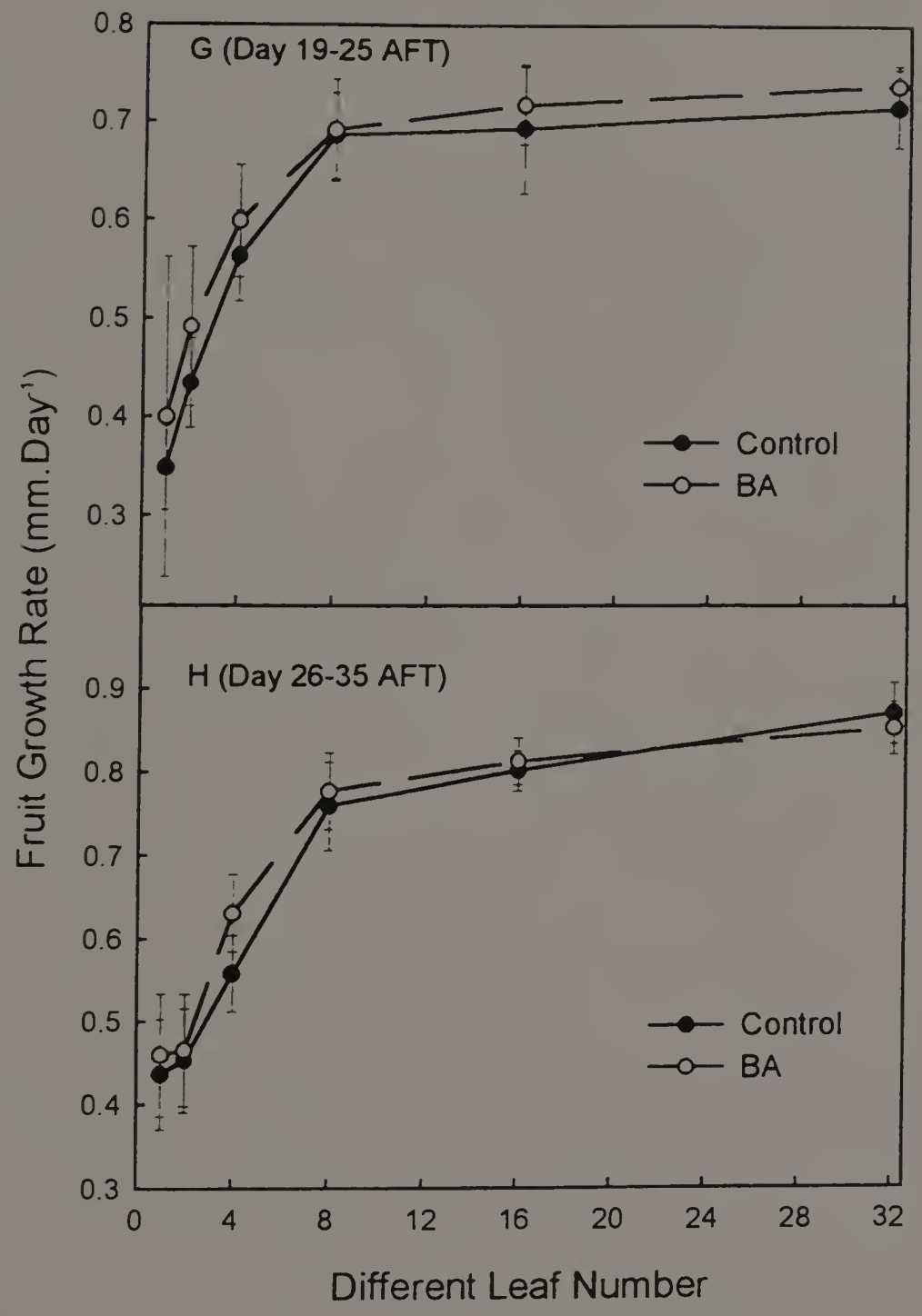

Figure 20. Effects of BA and Different Leaf Number Per Fruit on Fruit Growth Rate of 'McIntosh' Apples

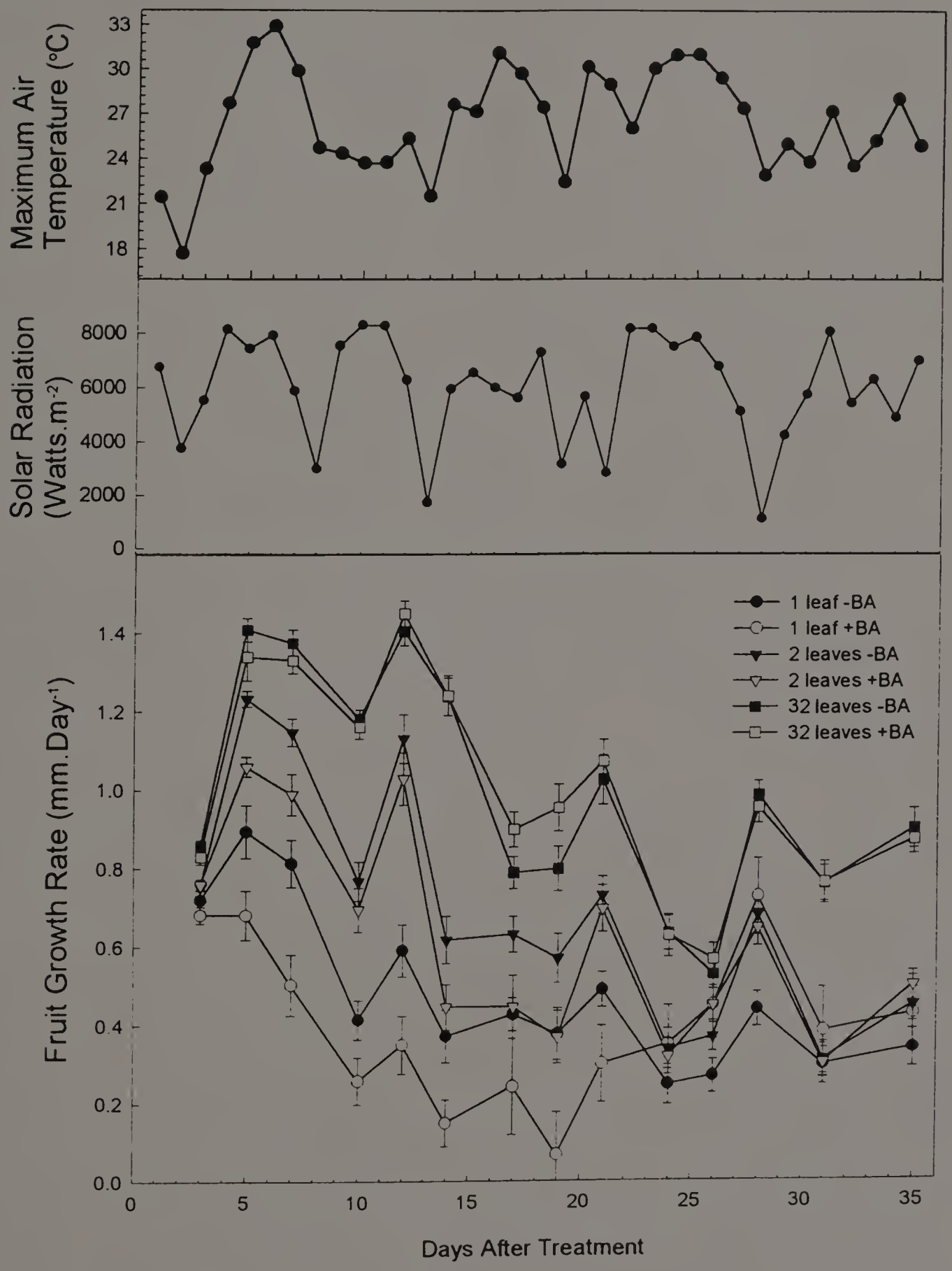

Figure 21. Effects of BA and Leaf Number on Fruit Growth Rate in 'McIntosh' Apples (1997)

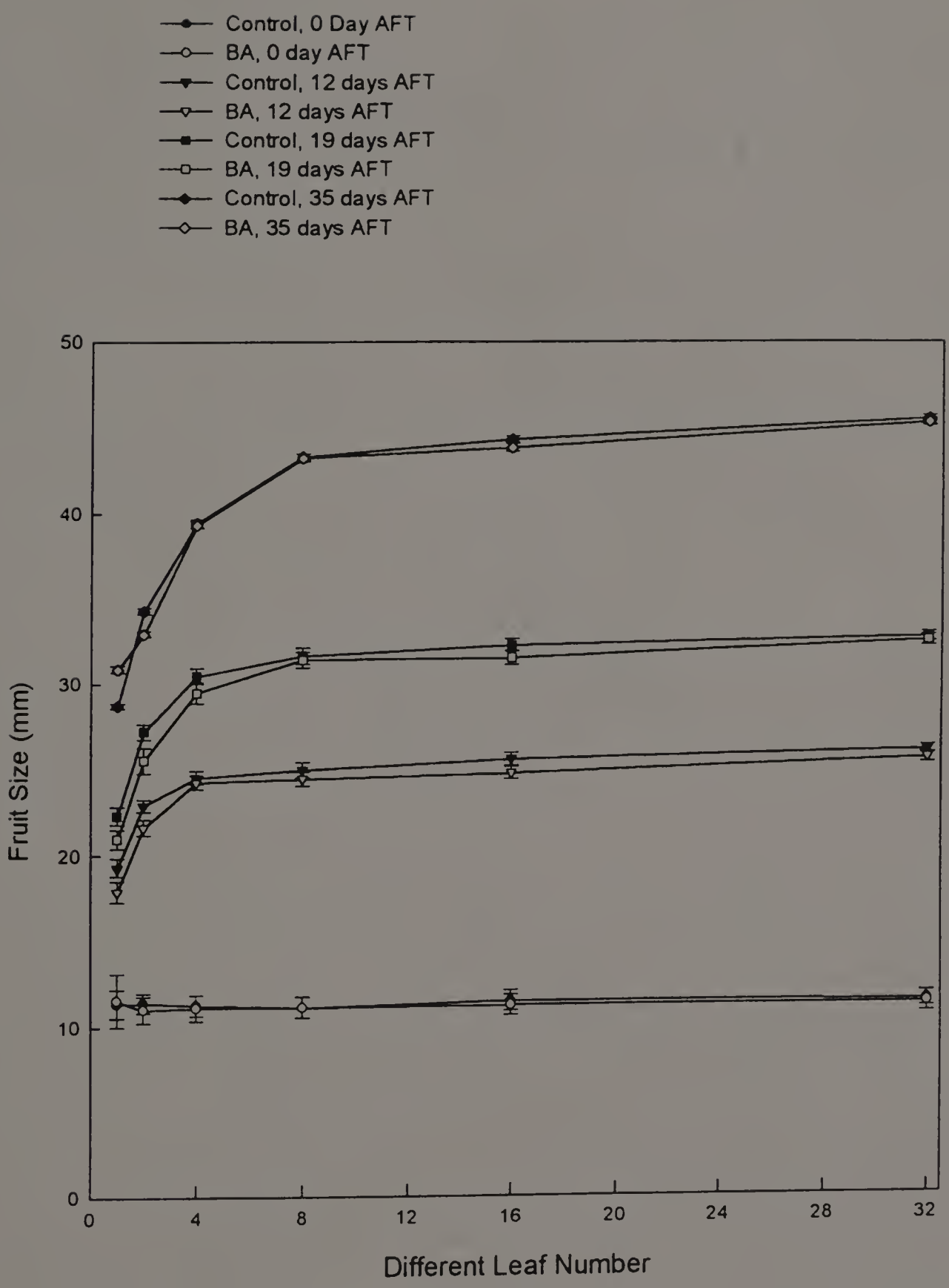

Figure 22. Effects of BA and Leaf Number on Fruit Size of 'McIntosh' Apples at Different Dates

CHAPTER V

DISCUSSION

BA Involvement in Fruit Abscission and ^{14}C-Assimilate Partitioning in Apples

Following initial set, energy produced by the photosynthesis in the leaves is required for young fruit to continue to grow and develop. Energy must be translocated from the leaves to the fruit. Schneider (1978) suggested that the mechanism of fruit thinning by NAA was due to interference with photosynthate transport from leaves to fruit since NAA decreased the amount of reducing sugars in the flesh of young fruit (Schneider and Lasheen, 1973) and reduced the translocation of sugars from leaves to the developing apple fruit (Schneider, 1975). Although Greene et al. (1992) showed that BA must come in contact with the leaves to thin, loss of carbohydrate translocation is unlikely to be the mode of action of fruit thinning. In this investigation, BA enhanced rather than reduced the translocation of ^{14}C-assimilate from spur leaves to fruit when applied directly to the 'McIntosh' apple fruit, but not when applied directly to the leaves (Figure 1 and 2). Similarly, BA promoted sugar movement from the foliage to the fruit in citrus (Mauk et al., 1986), cowpea (Adedipe et al., 1976), and grape (Skene, 1971). In 'Mala' cowpea, BA promoted the movement of ^{14}C-sucrose from the feeding leaves to the fruit and increased fruit set, whereas, in 'Adzuki' cowpea, which exhibits a relatively low young fruit abscission rate, BA had no significant effect on the translocation of ^{14}C-sucrose from the foliage to the fruit and also did not affect fruit set (Adedipe et al., 1976). Sorbitol and sucrose are the principal carbohydrates translocated in apple (Webb and Burley, 1962). Therefore, it is unlikely that BA thinned

apple fruit directly through restricting the translocation of photosynthates from leaves to fruit.

Effects of BA on Photosynthesis and Respiration in Apples

During the period of time following petal fall, fruit growth is occurring rapidly at the same time spur leaf area is increasing and shoot growth is rapid. There is a high demand for energy produced by photosynthesis of leaves at these multiple centers of metabolic activity (Corelli Grappadelli et al., 1994; Ferree and Palmer, 1982; Hansen, 1971; Quinlan and Preston, 1971; Tustin et al., 1992). Leaves on growing shoots do not export photosynthates until shoot growth exceeds 25-30 cm which is often 30 to 35 days after bloom (Corelli Grappadelli et al., 1994; Hansen, 1971; Lakso and Corelli Grappadelli, 1992). One can speculate that during this critical time relatively small changes in available carbohydrates may result in some fruit receiving insufficient carbohydrates to continue to grow, and thus they abscise. In this investigation, foliar application of BA reduced net photosynthesis of 'McIntosh' apple leaves by 10 to 15% (Figure 3 and 6). This suppression persisted for approximately 6 days depending on the weather, especially temperature, and resulted in a more than 50% reduction in fruit set when compared with nontreated control. Shading or application of photosynthetic inhibitors shortly after bloom, which decreased photosynthesis rates and thus reduced the carbohydrates available to the fruit, has been suggested as a primary factor responsible for increasing early fruit abscission of apple (Auchter et al., 1926; Byers et al., 1985; 1990 a, b, 1991; Cohen, 1991; Polomski et al., 1988; Beruter and Droz, 1991; Schneider, 1977, 1978; Doud and Ferree, 1980), peach (Byers et al., 1985; Delvalle et al., 1985), sweet orange (Moss, 1976), and litchi (Yuan and Huang, 1988). Schneider (1978) used shade cloth to reduce light interception of 'Delicious' apple trees by 25% for 2

et al., 1985), sweet orange (Moss, 1976), and litchi (Yuan and Huang, 1988). Schneider (1978) used shade cloth to reduce light interception of 'Delicious' apple trees by 25% for 2 days and caused a 90% fruit abscission compared with 66.1% of nonshaded controls. Byers et al. (1991) reported that even two days of artificial shade induced more apple fruit abscission than NAA, ethephon, or carbaryl + oil spray and shading the whole trees for three days caused 98% fruit abscission. They suggested that naturally occurring fruit drop in the period from 15 to 40 days after full bloom may be initiated by 2-4 days of cloudy weather. Stoper et al. (1997) found that foliar application of NAA caused 10 to 24% reduction in photosynthesis rates, and suggested that this reduction in photosynthesis was related to fruit thinning caused by NAA. Carbon balance models indicated a potential limitation of carbon availability during the first five weeks after bloom, a critical period for fruit set and fruit division (Lakso et al., 1989; Lakso and Corelli Crappadelli, 1992). Therefore, it is reasonable to assume that a 10 to 15% reduction in photosynthesis rates of apple leaves following BA application, sustained for several days, may be an important factor contributing to the early apple fruit abscission.

The amount of photosynthates available within the plant is related to net photosynthesis which is regulated by three factors (Pasian and Lieth, 1989). First is gross photosynthesis (P_g), which is the total amount of carbon fixed by the plant. The second and third, which consume some of the photosynthates, are photorespiration (R_p) and dark respiration (R_d). More simply stated $Pn=P_g-(R_d+R_p)$, which can be developed to $Pn+R_d=P_g-R_p$. Then $Pn+R_d=P$, where $P=P_g-R_p$. In this investigation, dark respiration of apple leaves was increased by foliar application of BA (Figure 4, and 7), which was in accordance with the results observed by Greene (unpublished data). In 1995 and 1996, there was no

significant difference in the sum of Pn and dark respiration among treatments and control (Figure 5 and 9), which meant that P did not change in value. Therefore, the reduced net photosynthesis following BA application mainly resulted from the increased dark respiration caused by BA application.

However, the response of dark respiration of apple leaves to BA application was temperature dependent. Dark respiration of apple leaves was increased when temperature following BA application was high (30°C) (Figure 7), whereas there was no effect when temperature was lower (20°C) (Figure 8). The temperature-dependence of dark respiration may explain why BA had no significant effect on photosynthesis and dark respiration of apple leaves on the sixth day after treatment in 1995, which was a cloudy cold day (Figures 3 and 4). This may also explain the erratic thinning observed following BA application. Stoper et al. (1997) reported that BA at 50 $mg.l^{-1}$ reduced photosynthesis rates by 10% in 'Redchief Delicious' but it was not at the level of statistical significance. However, they did not report the temperature when they measured. Further, the photosynthesis rates that they reported ranged from 12 to 15 $\mu mol.m^{-2}.s^{-1}$, which is relatively low when compared to approximately 20 $\mu mol.m^{-2}.s^{-1}$ reported by Schechter and Proctor (1994) and 18 to 19 $\mu mol.m^{-2}.s^{-1}$ in this investigation.

Fruit Thinning, Abscission, and Nonstructural Carbohydrate Status

BA has been an effective thinner on many apple cultivars (Bound et al., 1991b; Byers and Carbaugh, 1991; Elfving, 1989; Greene and Autio, 1990). The results we observed in three years confirmed previous reports that BA is an effective thinner on 'McIntosh' apples (Table 1, 2, and 4).

Generally there is more than one peak in apple fruit abscission during 'June Drop' (Abruzzese et al., 1995). In this investigation, there were two waves of fruit drop during 'June drop' period on 'McIntosh' apples regardless of BA application (Figure 18). These peaks appear to be innate but the peak value may be affected by weather. Foliar application of BA significantly increased the first peak, which occurred between 10 and 14 days after BA treatment. At this time, approximately half of then existing fruit abscised from treated trees in four days, whereas only one fourth abscised from the control trees. Therefore, BA appears to accentuate naturally-occurring waves of fruit abscission. Fruit growth usually reduced or slowed down 1 to 2 weeks before abscission in apples, therefore, at least 1 to 2 weeks are required for apple fruit from initiation of the process of abscission to actual abscission (Abruzzese et al., 1995; Magein, 1989). Thus, the time of application of BA is very important, since application made too far in advance or following a fruit abscission peak may result in insufficient thinning (Greene and Autio, 1989). Similarly, Byers et al. (1991) found that artificially shading for 2 to 3 days almost defruited trees when shaded 14, 21 and 28 days after bull bloom, but it did not reduce fruit set when shaded 8, 35, or 42 days after full bloom.

Carbon balance models suggested a potential limitation of carbohydrate availability during the time of early fruit development (Lakso and Corelli Grappadelli, 1992). Carbohydrates in 'McIntosh' apple leaves decreased dramatically over the 12 or 13 day observation period, confirming that carbohydrates available to developing fruit and growing bourse shoot were limiting (Figure 12 and 16). The carbohydrate contents were further lowered by BA application, indicating that the available carbohydrate supply for developing fruit and growing bourse shoot was further aggravated by BA application, thus causing

further stress and additional fruit abscission. Similarly, reduced soluble carbohydrate level in the leaves and a limited carbohydrate supply caused by increased respiration at high temperature or low light was suggested as a primary factor responsible for serious premature abscission of fruit in apple (Fukui et al., 1984 a, b; Kamakura, 1990; Kondo and Takahashi, 1987), cyathia in poinsettia (Miller and Heins, 1986), young pods of snap bean (Wittwer, 1954), and young bolls of cotton (Guin, 1976).

Our results showed that abscising fruit, regardless of BA treatment, contained higher carbohydrate concentration than persisting fruit (Table 6). Similar results were also observed by Abruzzese et al. (1995) and other researchers. Abscised or abscising apple fruit had higher calcium concentration, and lower dry weight, nitrogen, potassium, amino acids, and proteins than persisting fruit (Abruzzese et al., 1995; Howlett, 1923; Quinlan, 1969). Abscising citrus fruit had higher reducing and total sugars, and lower nitrogen, phosphorus and potassium than persisting fruit (Ruiz and Guardiola, 1994). The reason for high soluble sugar in abscising or abscised fruit was attributed to a reduction in their use (Abruzzese et al., 1995), since fruit growth reduced or slowed down 1 to 2 weeks before abscission in apple (Abruzzese et al., 1995; Magein, 1989) and citrus (Ruiz and Guardiola, 1994; Zucconi et al., 1978). Byers et al. (1991) reported that fruit destined to drop stopped growth from one to six days (21-26 days after full bloom) after a three-day period of artificial shade. Therefore, respiration rates, which positively related to rates of cell division and fruit growth, decreased with or before fruit growth slows (Darnell and Martin, 1988). Reduced respiration results in limited energy production which in turn reduces the synthesis of amino acids and proteins, and lowers amino acid and protein content in abscising or abscised fruit. At the same time, reduced respiration causes the accumulation of soluble sugars.

Polomski et al. (1988) reported that shading apple limbs for 5 days after full bloom reduced fruit set to half that of the nonshaded limbs, however, carbohydrate contents in the fruit on shaded and nonshaded limbs were similar. In this investigation, there was no significant difference in carbohydrate content of fruit of BA-treated trees and nontreated trees (Figure 13 and 17). This may be attributed to the sample containing some abscising fruit which usually have considerably higher carbohydrates, since about 83% of then existing fruit of BA-treated trees abscised during ' June drop' (Figure 18), and abscising fruit cannot be visually distinguished from persisting fruit until shortly before abscission.

In general, there is a positive relationship among seed number, fruit size, and fruit set. Seeds are a major factor affecting fruit set (Abbott, 1959; Dennis, 1986; Luckwill, 1948; MacDaniels, 1928) and growth (Denne, 1963; Dennis, 1986; Heinicke, 1917), since they are a rich source of hormones (Dennis, 1986; Luckwill, 1948). Fruit size increases with seed number (Denne, 1963; Dennis, 1986; Heinicke, 1917), and eight seeds are required to achieve maximum size of 'Delicious' apple fruit (Williams, 1977). Fruit which contain the fewest or weakest seeds are usually the first to drop (Childers et al, 1995; Heinicke, 1917; MacDaniels, 1928). In this investigation, abscising fruit during 'June drop' had lower total seed number than persisting fruit, regardless of BA treatment (Table 3). Although there is no correlation between seed hormone content and fruit set in apples (Crowe, 1958; Ebert and Bangerth, 1982), seeds or seed derived hormones affect fruit retention (Dennis, 1986) but presumably indirectly. High levels of hormones in the seed are thought to cause diversion of metabolites to the fruit and enable them to compete more efficiently with other growing organs of the plant (Crane, 1969). Therefore, fruit with more normal seeds have higher levels of hormones and thus higher metabolic activity or higher sink strength, leading to their

successful survival in the competition with other fruit, which have relatively lower seed number, and bourse shoot tip. The weaker sinks will lose out in competition with strong sinks, and finally abscise (Addicott, 1982). Therefore, more fruit with lower seed number were maintained when the metabolite supply, including carbohydrates, are ample. This situation occurs where spurs have a large leaf area (Heinicke, 1917), or trees have a light crop where leaf to fruit ratio is quite large (Denne, 1963; Schander, 1956). But fruit with a relatively high seed number were maintained when the metabolite supply is limited. This occurs when spurs are weak, small and have limited leaf area or when trees have a heavy crop load resulting in a low leaf area to fruit ratio (Denne, 1963; Heinicke, 1917).

Sorbitol, Bourse Shoot Tipping and Fruit Set

Injection of sorbitol solutions into branches increased fruit set on 'Delicious' apples (Dennis, 1986). Application of sucrose to bean explant retarded petiole abscission (Biggs and Leopold, 1957; Brown and Addicott, 1950). In this investigation, a foliar spray of 5% sorbitol had no effect on 'McIntosh' apple fruit set (Table 7). An alternative explanation is that the concentration may be inappropriate.

Fruit abscission is considered to be the result of competition among fruits, and between fruits and vegetative shoots for essential metabolites, including carbohydrates (Abbott, 1960; Knight, 1980; Quinlan and Preston, 1971). In this investigation, shoot tipping increased fruit set regardless of BA application (Table 8). BA thinned 'McIntosh' apple fruit regardless of whether bourse shoot tips were removed or not, and there was no interaction between BA and shoot tipping. These results can be explained in terms of source-sink relationship. A reduction in fruit drop by shoot tipping is attributed to more photosynthates moving into the fruit (Grauslund, 1978; Quinlan and Preston, 1971), since some sinks (shoot

tip) were removed. BA application, which reduced net photosynthesis as observed in experiment 3 and 4 and reduced carbohydrates available to developing fruit, promoted fruit abscission even when the shoot tip was removed. Therefore, the combination of BA application, which reduced a source of carbohydrates, and the presence of a shoot tip, which is a strong sink, resulted in less carbohydrates available to developing fruit and thus the least fruit set. BA thinned 'McIntosh' apple fruit even when the bourse shoot tip was removed, suggesting that BA thinned fruit not through promoting vegetative growth, at least of the bourse shoot.

Leaf Number, BA and Fruit Set

Photosynthesis in the leaves is the source of carbohydrates for fruit development (Addicott, 1982). Reduction in leaf area by removal of spur and shoot leaves, which reduces carbohydrates available to young fruit, causes an extensive fruit abscission in apples (Arthey and Wilkinson, 1964; Ferree and Palmer, 1982; Llewelyn, 1963, 1968; Quinlan and Preston, 1971). Ferree and Palmer (1982) found that final fruit set was reduced by a combination of ringing and removal of 50% of spur leaves, whereas a combination of ringing and removal of all spur leaves caused abscission of all fruit. In this investigation, about 25% of the fruit abscised when spurs were reduced to one fruit and one leaf on girdled fruiting branches, whereas no fruit abscised when the leaf number was greater than two on similar spurs (Table 9 and 10). This suggested that available carbohydrate was a limiting factor in apple fruit set, and at least two leaves are required to support a fruit to maturity under the condition occurring in 1997. BA thinned 'McIntosh' apple fruit only when one leaf was on the girdled fruiting branches, but not when leaf number was more than two (Table 9 and 10). The abnormally high fruit set on branches with low leaf number can be explained in terms of

source-sink relationships. Girdling prevented photosynthate from moving away from the girdle to other portions of the tree through phloem, and thus relieved competition that would normally occur from other fruit and growing shoots for carbohydrates and other metabolites. Apparently one leaf almost provided sufficient carbohydrates to meet the threshold demand for one apple fruit on the isolated branch. However, after foliar application of BA, which reduced net photosynthesis by 10 to 15% (Figure 3 and 6), the available carbohydrates were reduced below a threshold level causing abscission of some fruit. When more than two leaves were present on girdled branches, fruit had sufficient available carbohydrates to meet the threshold demand for continuing growth regardless of BA application.

Leaf area also has a strong influence on fruit growth. Fruit growth rate, fruit size and weight increased dramatically with increasing leaf number from one to eight, and then a steady increase thereafter (Figure 19, 20, 21 and 22, Table 11). Fruit growth rate was decreased by BA when less than 16 leaves were present on the girdled branches but only from 3 to 7 days after treatment. BA did not influence fruit growth when leaf number was 32 on the girdled branches. These results indicated that carbohydrate was a limiting factor in fruit growth and at least 8 leaves were required to support a fruit to grow normally in isolated branches.

The effect of BA on fruit growth rate was influenced by solar radiation and temperature (Figure 21). BA did not influence the fruit growth during the first three days after treatment, probably due to low temperature and solar irradiation. However, fruit growth rate on branches with one leaf treated with BA was significantly reduced when the temperature was high from 3 to 7 days after application. This is consistent with the results

we observed in experiments 3 and 4, that BA reduced photosynthesis through increasing dark respiration at high temperature, but not at low temperature.

Normal seed number was decreased, whereas aborted seed number was increased with increasing leaf number per fruit (Table 11), but total seed number was not affected (Table 11). Similarly, Byers et al. (1991) found that shading caused seed abortion in 'Redspur Delicious' apple fruits retained by the tree after three days of shading. He speculated that the seeds are quite small, but the intensity of demand for specific photosynthates could in turn abort seeds, thus triggering a process leading to fruit abscission.

Summary

The nonstructural carbohydrate contents in 'McIntosh' apple leaves decreased dramatically over the observation period, which confirmed previous reports that carbohydrate supply is limited at this stage after initial fruit set (Hansen and Grauslund, 1973; Lakso and Corlli Grappadelli, 1992), since there is a high demand for carbohydrates. During this period of rapid growth, increased fruit size is attributed primarily to the dramatic increase of cell number resulting from cell division (Denne, 1960, 1963; Dennis, 1986; Wismer et al., 1995). This rapidly increased cell division involves the synthesis of proteins (enzymes), hormones, DNA, RNA, lipids and other molecules. The synthesis of all these molecules requires a large amount of energy and building blocks provided by usual oxidative respiration of soluble sugars. At the same time, the bourse shoot grows quickly (Abbott, 1960; Quinlan and Preston, 1971; Ferree and Palmer, 1982), which also demands energy and building blocks. At this critical stage of development, fruit must compete with other fruit, and with the bourse shoot tip for metabolites, including carbohydrates (Abbott, 1960; Knight, 1980; Quinlan and Preston, 1971; Wardlaw, 1968). Weaker fruit, which have a relatively low seed number, will

be unable to compete with stronger sinks and thus abscise (Addicott, 1982). BA application reduced net photosynthesis which further reduced the carbohydrates available to developing fruit, thus leading to intensified competition among fruits , and between fruits and vegetative shoots, thereby causing more fruit abscission. However, after 'June drop', fruit growth is due mainly to the cell enlargement and carbohydrate demand is not that critical for fruit growth, thus carbohydrate supply has less influence on final fruit set.

BA did not affect the movement of ^{14}C-sorbitol from the leaves to the fruit when applied directly to the leaves, and it thinned 'McIntosh' apple fruit only when one leaf was on the girdled fruiting branches. This is interpreted to mean that BA thinned apple fruit not directly through affecting the movement of carbohydrates from leaves to fruit, but through increasing dark respiration and decreasing net photosynthesis, thus leading to limited carbohydrate supply, thereby accentuating fruit abscission.

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